THE

JOURNAL OF

COMPARATIVE PATHOLOGY

AND

THERAPEUTICS.

Vol. XLVI.—No. 2. JUNE 30th, 1933. PRICE 4s.

THE CONTROL OF CAMEL TRYPANOSOMIASIS.

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INTRODUCTION.

THIS paper is a record of work carried out in the Sudan since early 1927, the object of which has been to develop a routine system of camel trypanosomiasis control which can be generally applied in the field. The policy, therefore, has been for the laboratory to carry out preliminary or critical experiments and to follow these up wherever indicated by larger scale field work. No measure has been finally adopted unless it has been found practicable under local field conditions or until it has been modified to suit them. Notes on individual sections of the work have appeared in the Annual Reports of the Sudan Veterinary Service for the years 1927-1932 inclusive but, as it can now be claimed that a complete method of trypanosomiasis control has been evolved, in which little or no change is likely to occur, the publication of the work as a whole is indicated.

In regard to the term "camel trypanosomiasis," it must be explained that in the Sudan camels are not reared or worked in proximity to areas infested with tsetse flies, although occasions have arisen on which they have been introduced into such areas—with disastrous results. Thus, although camels appear to be susceptible to all the pathogenic trypanosomes, the species which normally attracts attention is *Tryp. soudanense*, which may be regarded

as merely a local strain of *Tryp. evansi*, and which is transmitted by tabanid flies. Certain small scale observations have been carried out on tsetse-borne trypanosomes, and these will be mentioned in the later portions of this paper.

Since the seasonal incidence of camel trypanosomiasis has a distinct bearing on the application of control methods, local climatic conditions must be briefly noted. The area in which camel trypanosomiasis is enzootic assumes the form of a " belt," approximately bounded by the thirteenth and fifteenth parallels of North latitude and extending from East to West across the whole country. Within this belt there is a single annual rainy season which lasts with slight variations from June to October, during which period the rainfall is usually between 10 and 20 inches. Tabanid flies which are rare in the dry season then increase greatly in numbers and camel trypanosomiasis assumes epizootic proportions. To the North of this zone there are many camels but the rainfall is less and there are practically no tabanid flies and consequently little or no trypanosomiasis ; to the South, conversely, the rainfall is heavier and more prolonged and tabanid flies are numerous throughout most of the year, but there are no camels. Furthermore, the specific danger is to working camels which have to remain in the infection zone, since the camel-owning nomads migrate to their more northerly grazing grounds during the rainy season.

Of the two broad alternative methods of control, namely, preventive and curative, the latter has now been entirely adopted. The appearance on the market of Naganol (Bayer 205) would have ensured this policy, even if otherwise it were not the only practicable one in the Sudan. As a curative agent for a specific infection or group of infections in a specific host, Naganol has no parallel in medicine, and the control of camel trypanosomiasis as now practised depends almost entirely on the proper administration of this compound.

Diagnosis.

The first essential, before adopting any curative treatment, is accurate diagnosis. Although such a postulate is obvious on general grounds, there have been two additional reasons for insisting on it while developing the treatment of camel trypanosomiasis with Naganol. First, to have administered the drug to non-infected camels in large numbers would have been very wasteful, and second, the subsidiary observations on relapses and reinfections following treatment would with faulty diagnosis have been based on the study of inaccurate data.

Until recently the diagnosis of camel trypanosomiasis under field conditions could only be attempted by microscopic examination of the blood. At its best, by examining cover-slip preparations of fresh blood, this method has been found to miss about 80 per cent. of infected camels if only a single examination is carried out, while

conditions of transport in the camel areas of the Sudan are often those from which one wishes to protect a microscope.

The Formol-Gel Test.

This test constituted the first attempt at developing an indirect method of diagnosis. It had previously been studied on a small scale in India and Morocco and had given promising results. Knowles (1924, 1925, 1927) in the Sudan studied it over a period of three years, and concluded that it constituted a very great advance on microscopic examination. The test has been sufficiently described by Knowles and no great discussion of the technique is now necessary. Briefly, however, it may be stated that the test consisted in mixing two drops of formalin (40 per cent. formaldehyde) with 1 cubic centimetre of serum from a suspected camel and allowing the mixture to stand for 24 hours. Complete gelation of the serum was taken to indicate infection with trypanosomes. The test, according to this technique, was in general use throughout the Sudan for three years (1927-1930), during which period records of every camel diagnosed and treated were collected in the laboratory. The final conclusions were that it was an extremely valuable test, but that it was too imperfect for complete reliance to be placed on it. The objections which were raised by veterinary officers were studied in the laboratory, and found in all cases to be justified. The general assessment of the test was that it was about 75 per cent. accurate as a single test applied on one occasion only. The failures or objections were classified into four main groups :—

(i) The length of time that must elapse between infection and positive reaction to the test was too long. The average was found experimentally to be about six weeks, but in too many cases it was much longer.

(ii) No reaction ever developed in some infected camels observed over periods of several months.

(iii) Too many camels giving positive reactions were not infected.

(iv) When performing the test many serum samples underwent partial gelation of various degrees, and such reactions could not be interpreted.

A statistical study of all objections was not made, as the mercuric chloride test was being simultaneously worked out in the laboratory and was showing promise of being so greatly superior to the formol-gel test that it was decided to give the latter no further attention.

The Mercuric Chloride Test.

It is not necessary to give more than a brief description of the principles of this test. They have already been set out in some detail in a first publication by Bennett and Kenny (1928) and later by Bennett (1929). Seeing, however, that the principle and, therefore, its practical applications, appear to have been misunderstood in certain quarters, some small elaboration may not be superfluous.

The main principle is similar to that of the formol-gel test and depends, as shown by Horgan and Bennett (1929), on the precipitation of excess of euglobulin in the serum of infected camels; the question of detecting "specific antibodies"—if such exist—is not at issue. The technique was evolved from the observation that, by choosing a suitable dilution, many soluble compounds would precipitate the excess. In the case of mercuric chloride, dilutions stronger than 1-20,000 would produce precipitates in the serum of all camels, whereas weaker solutions while having no effect on the serum of normal camels would regularly produce a precipitate in the serum of those infected with trypanosomes. In old established cases precipitation could be obtained with much weaker solutions, and in advanced cases even with distilled water. It was thus realised, as was subsequently proved by experiment, that in order to detect infection as early as possible it would be necessary to use the strongest solution of mercuric chloride that would not cause precipitation in the serum of normal camels. Extensive observations have shown that the strongest permissible solution is 1-25,000.

The method of applying the test is, therefore, to add one drop of suspected serum to 1 cubic centimetre of 1-25,000 aqueous mercuric chloride solution in a small test tube and to mix by gentle movement. The appearance of a white precipitate within a few moments indicates infection. It is thus immediately evident that, assuming equal accuracy, this test has two great advantages over the formol-gel test, the first being that a result is obtained immediately instead of 24 hours later, and the second that there can be no "partial reaction"; the density of the precipitate will vary, being heavier in the more advanced cases, but a precipitate of any degree constitutes a positive result.

General use of the test in the field for three years has shown that certain precautions must be observed, and these may now be noted.

The Mercuric Chloride Solution.

The sample of mercuric chloride must be pure.* Ordinary pharmaceutical samples are liable to contain small quantities of impurities, and a supposedly 1-25,000 solution may actually be more nearly 1-30,000, and will thus only detect cases in a later stage of infection; or, from the alternative standpoint, it may be possible to use a 1-20,000 solution of some (*i.e.*, impure) samples without fear of obtaining positive reactions in healthy camels. But, for use in the field, the solution should be prepared in a laboratory, since accuracy is essential and field officers lack the necessary facilities for ensuring it. Further, solutions should not be kept in stock for long periods, as they undoubtedly deteriorate. The reason for this deterioration has not been studied, but its effects are avoided by

* The only salt used in the Sudan is the British Drug Houses' "A.R." standard.

making monthly issues of fresh solution to all field officers. A convenient practical procedure has been to issue to each officer a special glass stoppered bottle (Pyrex glass), into which the fresh solution is poured after discarding the old.

The Serum.

The best results cannot be obtained unless the serum to be tested is clear. Under laboratory conditions no difficulty need be experienced, since any serum that does not separate perfectly from the clot can be centrifuged. The technique adopted in the field is to separate the clot from the tube in which the blood is collected as soon as the clot is firm. By the next morning there is nearly always enough clear serum for the test (one drop only is required). On some occasions, however, there is good separation of serum, but this contains some corpuscles. The presence of corpuscles in the serum will give rise to a faint false positive reaction, but it has been found by experience that unless the number of corpuscles is considerable these pseudo-positive reactions, which are always very faint, do not appear for some minutes after adding the serum to the solution, and most of them can be avoided by reading the result of the test almost immediately after setting up. This constitutes a slight modification of the original technique, whereby it was recommended that the test should be read 10 or 15 minutes after setting up. The original technique was worked out under laboratory conditions, and the interval was recommended because the precipitate, although it appeared immediately the serum was added to the solution, continued to increase in density for several minutes. The test has, however, to be used in the field, and while the above-mentioned disadvantage to it exists the occasions to which it applies are relatively few. In the first place, it is very rarely that a single drop of clear serum cannot be drawn into a pipette, and in any case the false reaction is so faint that in any but the very earliest cases of infection no confusion could arise. In practice, therefore, all reacting camels should be treated with Naganol, even if the reaction is suspected to be due to corpuscles in the serum ; the only loss is a dose of Naganol, whereas by not adopting treatment the camel might be lost. As far as can be ascertained, the number of false reactions amounts in practice to only 2 or 3 per cent.

The Serum Pipettes.

It may be objected that the term " one drop " of serum is not very precise. There is no doubt that for purely laboratory work it might be better to titrate more accurately. The test, however, has been developed for field use, and even if greater accuracy were indicated there would be a strong objection to using graduated capillary pipettes in the field ; the cleaning of them alone would be almost impossible. Actually, there is no need for extreme accuracy, as was shown in the original description of the test in 1928, and in

local field practice plain " Pasteur " pipettes drawn out from small bore glass tubing are used. A moderately intelligent Sudanese soon acquires the dexterity to draw them of fairly constant calibre so as to deliver drops of approximately equal size. They are made and cleaned in the laboratory and when subsequently used in circumstances in which there is any difficulty in cleaning them they are thrown away ; their cost is negligible.

Accuracy of the Test.

The expression " accuracy " as applied to a test of this nature denotes a quality that has many aspects, few of which can be assessed otherwise than in relative terms. The object is to detect infected camels, but one cannot expect a positive reaction on the first day of infection, nor would one expect it to disappear on the first day of cure. The main considerations are :—

(*a*) The length of time after infection before a positive reaction develops.

(*b*) Whether it develops in all infected camels.

(*c*) Whether it ever disappears spontaneously.

(*d*) Whether it always disappears after cure, or whether it constantly recedes below a certain residual titre.

(*e*) The length of time after cure for it to disappear, or recede to its minimum.

(*f*) Whether non-infected camels ever give positive reactions.

These factors must be considered separately, and wherever comparison is indicated the formol-gel test, as the next-best diagnostic measure, must be used as a basis.

(*a*) *The length of time after Infection before a Positive Reaction Develops.*—This question was partially considered in an early paper (l.c., 1929). With the standard technique it was shown that probably 50 per cent. of camels developed a positive reaction within a fortnight and nearly all camels within three weeks. More recent observations have confirmed this. Attempts have been made to use a 1-20,000 solution in the field, with the view of detecting infection at even earlier stages, but the results have shown that—under field conditions at any rate—one is with this dilution likely to get more " positive " reactions in healthy camels than is justified by the detection of early infections. With the 1-25,000 dilution 25 camels have at various times been studied in the laboratory with weekly tests following infection, and the intervals required for a reaction to develop have been :—

Two weeks or less	15 camels.
Three weeks	9 „
Four weeks	1 camel.
Over four weeks	Nil.

The average " incubation " period is thus less than half that of the formol-gel test.

It must be remembered that trypanosomes, especially in experimentally infected camels, may appear in the blood within as short a

time as a week or ten days after infection. That this has occurred in the laboratory does not lower the practical value of the test. It is, in fact, noteworthy that *in the field* over a period of three years, during which many hundreds of infected camels have been examined both by the microscope and the test, no camel with trypanosomes in the blood has been found negative to the test. The explanation of this observation is not that camels are not submitted to the test in the earliest stages of infection, since in the larger camel units routine periodic tests are carried out whether the existence of trypanosomiasis is suspected or not. The explanation undoubtedly is that following natural infection trypanosomes do not appear in the blood after so short an interval as is frequently recorded in the case of artificial infection.

(*b*) *Does a positive Reaction develop in all Affected Camels* ?—The evidence is very strong that a reaction always does develop. In laboratory experience of experimental camels and of camels submitted for diagnosis all infected individuals have given a positive reaction. The observations extend to over 60 infected camels. Under field conditions veterinary officers have been particularly asked to bring apparent exceptions to notice, but none have been reported. Failure to develop a positive reaction to the formol-gel test has frequently been recorded.

(*c*) *Does the positive Reaction ever disappear Spontaneously* ?—In the field it is impossible to obtain evidence on this point, and in the laboratory the number of camels that can be allowed to proceed to an extremely advanced stage of the disease is limited. A total of 13 camels has actually been allowed to reach a very advanced stage—or occasionally to die—before adopting treatment, but in no case did the reaction disappear. The formol-gel reaction sometimes did so.

(*d*) *Does the Reaction always disappear after Cure* ?—There is overwhelming evidence, both in the laboratory and from the field, that it does. In the laboratory, 19 cured cases have been kept under continuous observation, while many others submitted to treatment have been subsequently retested at various intervals ; in the field, 87 cured camels have been specially observed for disappearance of reaction. In all the above cases the reaction has disappeared—*i.e.*, the titre has not only receded but has reverted to normal. (One exception may possibly be made to this statement, for which see (*f*)).

(*e*) *Interval between Cure and Disappearance of Reaction.*—As might be expected, this is much more variable than the time necessary for a reaction to develop ; when infection occurs all camels are at a comparable stage in that they are all clean, whereas when treatment is adopted cases in all stages of infection are involved. In the laboratory, limiting consideration to camels under continuous observation, a total of eight have been submitted to weekly tests. The shortest period in which the reaction disappeared was four weeks

(two cases), and the longest 13 weeks (two cases); the remaining reactions disappeared at seven, eight, nine and eleven weeks (one case each). Evidence from the field is not so precise; 99 cured camels have been periodically retested but observations were combined with attempts at using a 1-20,000 dilution of mercuric chloride. With this dilution 79 were completely negative after three months, twelve were lost sight of, and the remaining seven continued to show alternately very faint and negative reactions. It has since been found that a 1-20,000 solution is too strong, and it may for practical purposes be accepted that the field records support the laboratory observations, namely, that while the interval between cure and disappearance of the reaction is variable, few or perhaps no positive reactors will remain at the end of three months.

(*f*) *Do any Non-infected Camels give Positive Reactions?*—The laboratory has records of over 300 clean camels, of which two only have given faint positive reactions; these two were subsequently lost sight of. In the field it is impossible to prove whether every reacting camel is infected or not, but only one positive reaction in a certainly free camel has come to notice. This beast gave a strong positive reaction to a routine test, although it was in first class condition and no trypanosomes were visible in the blood. Naganol was given, the camel remained in good condition, but the positive mercuric chloride reaction remained at its original strength for six months, after which period tests were discontinued. As the result of the foregoing evidence it is certain that a few non-infected camels give a positive reaction; to attempt an estimate of the proportion would be dangerous, but it is doubtful if they amount to 1 per cent. of the total. The proportion of healthy camels giving a positive formol-gel reaction is much higher.

In conclusion, in regard to diagnosis the mercuric chloride test has under field conditions confirmed the superiority to the formol-gel test of which it gave promise in the laboratory. Although it requires a rather higher standard of technique it is quicker and more accurate. From a purely economic standpoint, its greatest advantage is that it never, except in the "incubation" stage, which is of very short duration, misses an infected camel, whereas the formol-gel test, in addition to having a relatively long "incubation" period, misses a good many.

Tests with Dried Serum.

The common practice of storing standard samples of anti-toxic and other sera in the dry state suggested that a similar procedure might be possible in the case of camel serum destined to be submitted to the mercuric chloride test. The practical applications of the possibility are perhaps limited, but the climate in most of the camel areas of the world is suitable for preparing fairly dry samples of serum and for keeping them in a relatively dry state without much trouble. Experiments have shown that serum can be conveniently

dried on filter paper, and that after storage for some months positively reacting samples are still positive while negatively reacting sera remain negative. No attempt has been made to develop a precise technique, but the following points have been established :—

(i) The filter paper used must be of good quality and fairly hard ; otherwise, disintegration of the paper while carrying out the test will interfere with the readings.

(ii) In absorbing serum it is convenient to allow single drops to fall on to the paper at sufficient distances to avoid coalescence. For the tests, single drops can be cut out.

(iii) In carrying out the tests, the " drop " is cut into small pieces and soaked for at least half an hour in half a cubic centimetre of distilled water. Half a cubic centimetre of double strength mercuric chloride solution is then added. It is immaterial whether the pieces of paper be removed or allowed to remain. The reason for this procedure is that if the pieces of paper are placed directly into the test solution a positive reaction sometimes does not appear with known positive sera. It is assumed that such results depend on the degree to which the serum when fresh either penetrated the paper evenly or dried largely on the surface ; in the former case it would seem that the usual precipitate is formed but that it remains in the substance of the paper.

(iv) Samples of dried positive serum that have been stored for some time (months) have to be left in contact with the test solution for perhaps half-an-hour before the reaction develops.

It is not intended at present to proceed further with this form of test, but its practicability may at least be noted.

Treatment.

Since the introduction of " Naganol " (Bayer 205), research on the treatment of camel trypanosomiasis has resolved itself almost entirely into a study of this drug. The earlier work of Knowles (l.c. 1927) had unequivocally established that a single intravenous dose of 10 grammes was safe and would effect a certain cure, and further experiments on a preliminary scale by the same worker had suggested the possibility of reducing the dose. Nevertheless, it was decided not to proceed immediately to the determination of the minimum curative dose, the attitude being that, secure in the knowledge that a cure was always effected by 10 grammes, it would be better to retain this large dose in routine field practice in order to obtain large scale information on immunity ; to have used a minimum curative dose, the efficacy of which had not been proved by very extensive observations, would have been to risk the confusion of relapses with re-infections. It was not until the questions of immunity and prophylaxis were settled that any attempt was made to reduce the routine dose. It was further realised that in trying to establish the minimum curative dose, although a good deal of information might be made available from outside sources, it would be

necessary to confirm it locally. Experiments in the laboratory were not contemplated, since these would necessarily be on a very small scale, no principle was involved, and any result that might be obtained would have to be repeated with no modification of standpoint in the field. Having regard to Sudan conditions, in which it is often impracticable to give more than a single dose and a treated camel may not be available for reinspection for several months, the only safe way of establishing a minimum dose was to work downwards from a safe one and to treat a large number of camels at each step.

When ultimately wishing to reduce the dose it was rather surprising that more precise information was not obtainable from outside sources. However, it had been shown on a small scale by Knowles (l.c. 1927) that a single intravenous injection of 4 grammes, and possibly of only 3 grammes, of Naganol *together with* 2 *grammes of tartar emetic* could effect a cure. The few experiments recorded by him were not controlled by the injection of similar doses of Naganol alone ; and with the knowledge that a single dose of 2 grammes of tartar emetic is of no practical value, it seemed possible that 3 or 4 grammes of Naganol alone might be sufficient. Ilowaisky and Zeiss (1923), dealing with " Su-auru," which is probably the same disease, had shown that 3 grammes was probably not sufficient as a single dose, but instead of trying a larger single dose they proceeded to a repetition of small ones. Herzog and Lavier (1923) treated one case of " Debab " with a single intravenous injection of 4 grammes and effected a cure. A number of later papers, recording what at best can only be considered preliminary experiments, have come to notice, but the only record of large scale trials of a single relatively small dose is by Williams (1930). Unfortunately in this case no details are given, but it is stated that the mercuric chloride test is used for diagnosis and " All cases [all in India ?] so diagnosed are treated immediately with 4 grammes of Naganol." Thus it seemed that three grammes as a single dose might not be sufficient, while there was considerable evidence that 4 grammes was.

In order to obtain indisputable evidence on a large scale, the collaboration of two veterinary inspectors was sought. One of these was to treat 50 naturally infected camels with a single dose of 4 grammes and, as a reserve in case the 4 grammes dose should not prove universally successful, the other was simultaneously to treat 50 camels with a single dose of 7 grammes. The conditions of observation were laid down with some precision. In each case the 50 selected camels had to be such as could be kept at work and under continuous observation after treatment ; diagnosis was to be effected by detection of trypanosomes in the blood wherever possible, but failing this a positive reaction to the mercuric chloride test could be accepted ; following treatment each camel was to be examined at monthly intervals for at least three months, and longer if possible ;

at each examination the mercuric chloride test had to be applied and supplemented if practicable by microscopic examination of the blood. A camel was to be considered cured if it regained and/or maintained good bodily condition in work, if no trypanosomes were seen in the blood after the treatment, and if the positive reaction to the mercuric chloride test either disappeared (in the case of camels which could be kept under prolonged observation) or became progressively weaker (in the case of camels which could not be kept under such prolonged observation). Forty-nine camels which had received 7 grammes were thus kept under observation for six months, and all were definitely cured. Of the 50 camels which had received 4 grammes 12 were lost sight of after the third month, up to which time they appeared to be cured according to the specified conditions, although in some of them the mercuric chloride reaction had not yet become completely negative. The remaining 38 were considered completely cured. Since trying out these special treatments, over 1,500 infected camels over a period of about two years have been given a single intravenous injection of 4 grammes. No special records have been kept, but as nearly all of the patients have been officially-owned camels their subsequent histories are known. No report of a relapse has yet been received.

In regard to the curative dose of Naganol, there is thus no doubt that a single intravenous injection of 4 grammes is sufficient ; the possibility of using an even smaller dose is of no particular academic interest, but it will have to be considered from an economic point of view.

Before finally abandoning the question of the minimum curative dose, it is essential to discuss the apparent failures that were formerly recorded with much larger doses of Naganol than are now found consistently to effect a cure. In the Sudan, which alone may be considered because conditions have been strictly comparable, Knowles (l.c. 1927), records occasional failures with single doses of 4, 6, 8 and even 10 grammes. There is no doubt that in the earlier years of this drug's history it was, if not always different from modern samples, at least more variable. Experience in the constant handling of successive samples, almost from the earliest days of its existence, leaves one in no doubt that its physical properties at any rate have altered. Further, in equine therapy, which is not within the scope of this paper, one has in recent years found Naganol to be a relatively safe drug to use, whereas a few years ago its injection was liable to be followed by very disturbing symptoms. These observations, although in no way responsible for the delay in attempting to establish a minimum curative dose, would have justified a repetition of any earlier work.

(*To be continued.*)

CANINE DISTEMPER. THE HIGH ANTIGENIC VALUE OF THE VIRUS AFTER PHOTODYNAMIC INACTIVATION BY METHYLENE BLUE.

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In the course of previous work on the photodynamic action of methylene blue on certain viruses (Perdrau and Todd, 1933[1], [2]) it was found that a number of these were highly sensitive and were inactivated within a few minutes by concentrations of the dye of 1 in 100,000, under suitable illumination. Experiments have since been carried out to test the antigenic value of such inactivated viruses and it is the object of this paper to record the results obtained in the case of the virus of distemper.

At the time the present investigation was started—during the summer of 1932—the researches of Dr. P. P. Laidlaw and Major G. W. Dunkin on behalf of the Field Distemper Fund had just concluded and we had therefore at our disposal, though necessarily on a smaller scale, the excellent organisation (isolation accommodation, trained staff, etc.) which had been built up by them. For these facilities, for the strain of virus used, and last but not least, for their help and advice we are deeply indebted to our colleagues. Our chief regret is that existing conditions have necessarily limited the number and scope of the observations, especially as regards dogs.

Methods.

The methods used were those which have already been described in a previous communication (Perdrau and Todd, 1933[1]). Briefly, these consisted in exposing a suitable mixture of the virus with methylene blue—the latter in a concentration of 1 in 50,000 or 1 in 100,000—for a stated time (usually 30 minutes) to the light of a 100 c.p. pointolite lamp at a distance of 20 cm., in a layer of 2 mm. deep. Unless otherwise stated the virus preparation consisted of the supernatant opalescent fluid obtained after lightly centrifuging (for 10-15 min.) a two per cent. suspension of virulent spleen in normal saline, the spleen having been thoroughly ground with powdered quartz.

The greater part of the work recorded here has been done on ferrets and only a few experiments have been carried out on dogs owing to the time and expense involved in breeding these under such conditions of strict isolation that their susceptibility shall be unquestioned.

The temperatures of all the experimental dogs were regularly recorded. Ferrets contracting the disease were not allowed to die

but were killed with chloroform at the height of the disease and their spleens utilised as a source of virus for further experiments.

It will be assumed in the course of this paper that the work of Laidlaw and Dunkin on canine distemper is familiar to the reader, particularly as regards the experimental disease in dogs and ferrets and the immunisation of these animals.

Minimum time required for the inactivation of the virus.

In a preliminary experiment it had been found that the virus was inactivated in 30 minutes under the conditions already stated. The minimum time for inactivation was determined by exposing to the pointolite lamp for varying times mixtures of equal parts of a solution of methylene blue (1 in 50,000) and of the supernatant fluid from a 2 per cent. centrifuged saline suspension of infective ferret spleen. After irradiation the mixture was tested on ferrets, 1 c.c. being given subcutaneously.

TABLE I.

Minimum Time Required for the Inactivation of the Virus.

Ferret Number.	*Time of Exposure.*	*Result.*
F.90/32	5 minutes	+
F.91/32	10 „	0
F.92/32	20 „	0
F.93/32	Control mixture kept in the dark for 40 minutes	+

Note. + = clinical attack of distemper. O = no symptoms.
These symbols will be used throughout this paper with the same meaning.

An exposure of about 10 minutes therefore represents the minimum time required for the inactivation of the virus under the conditions of the experiment.

The two ferrets which survived from this experiment were tested with living virus 24 days after the original inoculation and were found to be immune.

The fact that such a small dose of inactivated virus had protected these ferrets against the subsequent inoculation of living virus was thought of sufficient interest to justify further investigation and a repetition of the experiment on a slightly larger scale.

Protection of the Virus by Living Cells.

A number of ferrets were injected, in duplicate, with mixtures of virus and dye which had been irradiated for periods of 10, 15, 20 and 30 minutes: two control animals receiving the virus only. The result of this experiment was entirely unexpected, as all the animals succumbed to the unmodified disease within 12 days.

A search for some explanation of result of this experiment revealed the fact that the spleen used as the source of virus had been collected the previous day : whereas in the first experiment the spleen from which the virus suspension had been prepared had been collected six days previously and had been kept frozen during that time. In view of our previous work on the protection afforded to some viruses against the photodynamic action of methylene blue, the most probable explanation of this result was that the tissue cells were still alive in one case and therefore capable of protecting the virus against the photodynamic action of methylene blue. In the first experiment this protective action of the tissue cells had presumably disappeared through the death of the cells.

The experiment was therefore repeated in exactly the same way as before with the identical spleen as the source of the virus, but instead of being comparatively fresh as on the first occasion, the spleen had by then been kept frozen for a period of 14 days and had at the end of that time been transferred for another three days into 75 per cent. glycerine in saline in order to ensure the death of the tissue cells. Here the results were very different, as all the ferrets which received the irradiated mixtures not only showed no symptoms of the disease but on subsequent inoculation with the active virus proved to be immune. The potency of the virus used in these inoculations was demonstrated by the development of the usual train of symptoms in control animals. The results are shown in Table II. The mixtures irradiated in this test consisted of equal volumes of 1 in 50,000 solution of methylene blue and of the supernatant layer of a centrifuged 2 per cent. suspension of infective ferret spleen.

TABLE II.

DIFFERENT BEHAVIOUR OF THE VIRUS TO THE PHOTODYNAMIC ACTION OF METHYLENE BLUE WHEN PRESENT IN LIVING AND IN DEAD SPLENIC CELLS.

Time of Exposure.	*Spleen One Day Old.*		*Same Spleen 17 Days Old.*		
	Ferret Number.	*Result after Irradiation.*	*Ferret Number.*	*Result after Irradiation.*	*Result of Immunity Test on 18th Day.*
10 minutes	F.103/32	+	F.113/32	O	Immune
10 „	F.104/32	+	F.114/32	O	„
15 „	F.105/32	+	F.115/32	O	„
15 „	F.106/32	+	F.116/32	O	„
20 „	F.107/32	+	F.117/32	O	„
20 „	F.108/32	+	F.118/32	O	„
30 „	F.109/32	+	F.119/32	O	„
30 „	F.110/32	+	F.120/32	O	„
Virus Controls not irradiated	F.111/32	+	F.121/32	+	
	F.112/32	+	F.122/32	+	
			F.134/32		+

The dose used for the tests was 1 c.c. of the mixture injected subcutaneously into ferrets.

The results in these experiments are entirely in accordance with those obtained with certain other viruses (Perdrau and Todd, 1933[2]), and there can be little doubt that failure to inactivate the *fresh* spleen emulsion in the experiment recorded above was due to the protective action exercised by living cells present in the fluid. If this view is correct it should be possible to abolish the protective action in a suspension of *freshly removed* infective spleen by the complete removal of all cells or cellular fragments. This was realised by filtration through a permeable collodion membrane, the filtration being very kindly made for us by Dr. W. J. Elford through one of his " Gradocol " membranes.

A freshly removed spleen from a ferret killed at the height of the disease was used as the source of the virus and a 2 per cent. suspension of this was centrifuged at high speed for two periods of 30 minutes each. A stained preparation of the small amount of deposit obtained on the second occasion appeared to be free from cells, as none were seen under the microscope. A portion of the supernatant fluid from the second centrifuging was set aside and the remainder was filtered through a collodion membrane of average pore size of 1μ. Samples of the unfiltered supernatant and of the collodion filtrate were separately mixed with equal quantities of 1 in 50,000 methylene blue and the mixtures exposed to the pointolite lamp for 30 minutes. The results are given in Table III.

TABLE III.

Protection of the Virus by Living Cells or Cellular Fragments.

Ferret Number.	*Dose.*	*Result.*
	Unfiltered Supernatant.	
F.123/32	1 c.c. of irradiated mixture	+
F.124/32	0·1 c.c. of untreated virus. Control	+
	Filtrate.	
F.125/32	1 c.c. of irradiated mixture	O
F.126/32	0·2 c.c. of untreated filtrate. Control	+

The result of this experiment leaves no doubt that, after the complete removal of all cells from a suspension of a freshly removed infective spleen, the virus is perfectly susceptible to the photodynamic action of methylene blue. It also shows that a very thorough centrifuging, as usually carried out in ordinary centrifuge tubes, does not necessarily remove all traces of tissue capable of protecting the virus.

The protective action of living cells on the virus has been gone into in some detail in view of its importance as a possible, and in fact the most probable, source of error in experiments of the kind described in this paper.

It may be noted here that, in the case of distemper, protection has never been observed with spleens which have been stored—either frozen or otherwise—for a week or more after removal from the ferret.

Antigenic Value of the Inactivated Virus.

In the experiments recorded so far, of the eleven ferrets which had received the inactivated virus, one died from an abscess in the throat before it could be tested with the living virus. The remaining ten animals were tested with living virus 14 days after the original inoculation and all remained well, showing that they had been satisfactorily immunised. The test dose of virus varied from 0·25 c.c. to 1 c.c. of a 1 per cent. uncentrifuged suspension of infective spleen. In order to test the adequacy of this test dose, control animals were injected with one tenth of this amount and all succumbed to the disease after the usual incubation period (9-10 days).

Some experiments were next made to determine : (1) the smallest effective immunising dose of the inactivated virus in terms of minimal infective doses of the virus before inactivation ; (2) the effect of prolonged irradiation on the antigenic value ; (3) the keeping properties of the irradiated mixtures, especially after drying.

Minimal Immunising Dose of the Inactivated Virus.

In order to find the minimal immunising dose, the virus preparation itself and another sample of the same preparation which had been inactivated under the usual conditions were titrated on two small series of ferrets. The supernatant of a thoroughly ground and

TABLE IV.

Minimal Immunising Dose of the Inactivated Virus.

	Ferret Number.	*Dose.*	*Result.*	*Result of Immunity Test 14 Days Later.*
Inactivated mixture	F.162/32	1 c.c.	O (throat abscess)	Died 9 days later from throat abscess. No signs of distemper.
	F.163/32	1 c.c.	O	O
	F.164/32	0·1 c.c.	O	O
	F.165/32	0·1 c.c.	O	+
	F.166/32	0·01 c.c.	O	+
	F.167/32	0·01 c.c.	O	+
Virus Controls	F.168/32	0·1 c.c.	+	
	F.169/32	0·01 c.c.	O	+
	F.170/32	0·001 c.c.	O	+
Control to immunity test. (1/10 of test dose) F.5/33				+

centrifuged suspension of infective ferret spleen (2 per cent.) in saline was used. An equal volume of 1 in 50,000 methylene blue in saline was added to the virus suspension in one case and an equal volume of saline in the case of the virus titration. The quantities used in the two cases are given in Table IV, the original amount of virus present in the two preparations being the same (i.e. a half dilution of the supernatant of a centrifuged 2 per cent. suspension of infective spleen).

The results obtained in this experiment are particularly interesting as showing the small amount of the inactivated virus which appears to be required for the production of immunity, since only 100 minimal infective doses of the virus (and probably a smaller number) were sufficient, after inactivation, to protect a ferret against a subsequent inoculation of at least ten minimal infective doses of the living virus.

It would seem probable that the dose of inactivated virus used in the earlier experiments (i.e. 1 c.c. of the supernatant fluid of a centrifuged 1 per cent. suspension of spleen) must have been very near the minimal immunising dose, since in the above test only one out of two ferrets was immunised with one tenth of that amount.

In a second experiment of the same type, where the source of virus was a ferret spleen which had been kept in the freezing chest (—10° C.) for 28 days, a similar dose of vaccine to that used in the first experiment failed to protect either of two ferrets. The virus content after 28 days must have been lower than on the first occasion, when the spleen which provided the virus had been kept for nine days only. This degradation of the virus content on keeping has been noted by Laidlaw and Dunkin.

Effect of over-irradiation on the Antigenic Value.

While the minimum time of irradiation necessary for the inactivation of the virus, under the usual conditions, is known within fairly narrow limits, it is advisable in practice to irradiate for a somewhat longer period so as to provide for possible uncertainties (e.g. variations in the virus content, in the intensity of the illumination, etc.) and, in order to determine whether this prolongation of the time of irradiation has any material effect on the antigenic value, two experiments were made.

In the first of these the mixture exposed to the pointolite contained the usual quantity of virus and of methylene blue (1 in 100,000). Samples of 1 c.c. were taken after various periods of irradiation and tested on ferrets. The tests for immunity were carried out at varying intervals and in the case of the standard exposure (30 min.) larger volumes of the irradiated mixture were given to two additional ferrets in order to see whether the virus had been completely inactivated in that time. The results are given in Table V.

TABLE V.

EFFECT OF PROLONGED EXPOSURE TO THE POINTOLITE LAMP ON THE ANTIGENIC VALUE.

Ferret Number.	*Time of Exposure.*	*Dose of Mixture.*	*Result after Irradiation.*	*Result of Immunity Test.*
F.138/32	30 minutes	1 c.c.	O	Immune 15 days later.
F.146/32	„	9 c.c.	O	Immune 15 days later.
F.147/32	„	7·5 c.c.	O	Immune 50 days later.
F.140/32	1 hour	1 c.c.	O	Immune 15 days later.
F.142/32	2 hours	„	O	Immune 15 days later.
F.144/32	4 hours	„	O	Immune 15 days later.
F.145/32	„	„	O	Immune 5½ months later.

The immunity tests on the various dates were all controlled by the inoculation of normal ferrets with the same or smaller doses of the living virus used in the case of the immunised animals.

It is clear from the above test that a prolongation of the period of irradiation to 24 times the minimum time necessary for inactivation (or eight times that used as a routine in this investigation) had no material effect on the antigenic value of the irradiated mixture, so that the risk of failure owing to over-irradiation is not great.

The second experiment was made with direct sunlight. A mixture of virus suspension and methylene blue was prepared as in the former experiment, with the single exception that both the virus and the dye were in double the concentration used on the previous occasion. This mixture was exposed to direct sunlight early in the day on the 14th of March of this year for 30 minutes. A rough measurement by photometer showed the light to be very approximately some 80 times that of the pointolite lamp at 20 c.m. The mixture under irradiation was covered as usual with a sheet of glass so as to exclude the ultra-violet rays. The result is given in Table VI.

TABLE VI.

EFFECT OF OVER-IRRADIATION WITH SUNLIGHT ON THE ANTIGENIC VALUE.

Ferret Number.	*Dose of Mixture.*	*Result after Irradiation.*	*Result of Immunity Test 14 days later.*
F.36/33	1 c.c.	O	+
F.37/33	8 c.c.	O	First symptoms 10th-13th day—recovery.
Control to immunity test—¼ of test dose — F.40/33			+

A prolonged exposure to a powerful source of light—in this case a not very bright sunlight—is thus clearly detrimental to the antigenic value, since a dose of the mixture so irradiated, and representing at

least eight times the minimal effective dose of pointolite-inactivated mixture, affords only a partial protection. The amount of irradiation in this case was, however, very high, probably of the order of two or three hundred times the minimum amount required for inactivation.

Keeping Properties of the Inactivated Virus, especially after Drying.

The supernatant fluid of a centrifuged 2 per cent. suspension of infective ferret spleen in saline was mixed with concentrated methylene blue to yield a final concentration of 1 in 100,000 and was irradiated as usual for 30 minutes under the pointolite lamp. Three separate batches of vaccine were so prepared and distributed into ampoules in quantities of 5 c.c. and submitted to the following procedures :—

(*a*) One batch was dried over sulphuric acid for a few days and then over phosphoric anhydride.

(*b*) A further batch was dried over phosphoric anhydride and the desiccator evacuated and filled with oxygen-free nitrogen and then re-evacuated.

(*c*) A third batch was kept fluid as prepared, in an atmosphere of hydrogen in an anaerobic jar.

The jar and the desiccators were kept in the dark in the cold room at a temperature of 0°—5° C. and the batches tested at various times. The results are given in Table VII. For these tests the dried vaccine was redissolved in distilled water. In testing the immunity of the ferrets which had received the vaccine the dose of virus used was 1 c.c. of the supernatant of a 1 per cent. suspension of infective ferret spleen. All the controls to the various immunity tests reacted in the normal way.

TABLE VII.

Keeping Properties of the Inactivated Virus.

Ferret Number.	*Nature of Vaccine.*	*Age of Vaccine.*	*Dose in terms of Minimal Standard Dose.*	*Result of Immunity Test 14 Days Later.*
F.8/33	Dried over H_2SO_4, then P_2O_5	62 days	5	Immune
F.9/33		„	5	Immune
F.10/33	Dried over P_2O_5	9 days	5	Immune
F.11/33		„	5	Immune
F.12/33	Fluid — in atmosphere of hydrogen	63 days	5	First symptoms 7th-9th day, then recovery
F.13/33		„	5	Immune

It is clear from the table that satisfactory vaccine which is dried immediately in the dark and in the cold preserves its antigenic properties quite well for a period of at least two months.

The vaccine kept in a fluid condition had obviously deteriorated on keeping but, as no special precautions had been taken to ensure its sterility and no antiseptic had been added, this is not surprising.

Immunisation of Dogs.

The good results obtained in the immunisation of ferrets with virus inactivated by the photodynamic action of methylene blue had as a natural corollary similar experiments on the dog, but here we were confronted with the almost insuperable difficulty of a supply of dogs whose history from birth was sufficiently well known to exclude with certainty the possibility of a previous attack of distemper, however mild. The expense of providing such a supply is now well known from the work of Laidlaw and Dunkin, and the high standard which they set in this particular respect must stand in future experiments with the virus of distemper in dogs. A similar high standard is not essential in the case of ferrets since in these animals distemper is almost invariably a fatal disease.

A few susceptible dogs were left over at the Farm Laboratories from the work of the Field Distemper Fund and these were put at our disposal by Dr. Laidlaw and Major Dunkin. The few experiments which it was possible to carry out with these animals are reported here but, owing to their small number, must be considered as of an entirely preliminary nature. They will, however, be continued as soon as susceptible animals are again available.

In the first experiment two dogs received a vaccine prepared in a similar manner to that employed in the case of ferrets, but the source of virus was the spleen of a dog killed at the height of an attack of distemper, the vaccine consisting of the inactivated supernatant fluid of a centrifuged 2 per cent. suspension. The virus controls to the two sets of inoculations were carried out on ferrets in order to preserve the small stock of dogs. The results are given in Table VIII.

TABLE VIII.

Immunisation of Dogs with Inactivated Dog Virus.

Dog Number.	*Dose of Mixture.*	*Result of Immunity Test 10 Days Later with Dog Virus.*
D.1	20 c.c. of inactivated 2 per cent. supernatant	O
D.2	2 c.c. do. do.	O
	Ferret control to immunity test. 1/10th test dose. F.14/33	+

The result of this experiment in which such a small amount as 2 c.c. of the supernatant fluid of a 2 per cent. suspension of infective dog spleen had, after inactivation, immunised a good-sized half-grown dog led us to an attempt to discover the minimal immunising

dose in the next experiment, but a different spleen was now used as the source of the virus and the outcome was that 9 c.c. of a similarly prepared vaccine failed to immunise a dog. It appeared that the spleen used in the first experiment had been an exceptionally good one from the point of view of virus content while that used in the second experiment was much poorer in that respect.* These facts were naturally not known at the time of the experiment. Lack of animals has so far prevented a repetition of the test.

It is well known from Laidlaw and Dunkin's work that the ferret spleen as a source of virus is inefficient after treatment with formalin for the immunisation of dogs unless repeated injections are given. The next experiment was therefore designed to test the value of ferret virus after inactivation with methylene blue for the immunisation of a dog. The vaccine was prepared in exactly the same way as that used in the first experiment on dogs except that the spleen was that of a ferret and the suspension was of 3 per cent. concentration instead of 2 per cent. This dog showed no immunity to a subsequent inoculation of living virus—a result in keeping with the experience of Laidlaw and Dunkin in the case of formolised vaccine. The experiment could not be repeated owing to lack of susceptible animals and in fact all experiments involving dogs have had to be discontinued for the same reason.

Discussion.

The principal point of interest which arises from the experiments recorded in this paper is the fact that a virus inactivated by the photodynamic action of methylene blue preserves its antigenic properties and can therefore be used as a highly efficient vaccine.

That this observation is not capable of general application is shown by the fact that in a few preliminary experiments, made both with neurovaccine and with calf lymph, no trace of immunity was observed in rabbits which had received a subcutaneous injection of a comparatively large dose of the inactivated virus.

In the case of the virus under consideration—that of canine distemper—the most striking feature in these experiments is the small dose of inactivated virus which can protect efficiently against a subsequent inoculation of living virus. One hundred minimal infective doses of virus, after inactivation, protect a ferret.

With formolised virus the dose given by Laidlaw and Dunkin represents 0·1 grm. of whole spleen pulp, which before formolisation they found to be always infective for the ferret at a dose of 0·00001 grm. The effective dose of formolised vaccine contains therefore at least 10,000 minimal infective doses of virus—that is one hundred times that of an effective dose of the methylene blue vaccine. Another point is that in the latter the greater part of the splenic tissue is removed, thereby eliminating the local swelling and somewhat

* After preservation in the frozen state for a period of two months this spleen tested on three ferrets proved non-infective.

persistent induration which may occasionally be observed at the site of inoculation in animals receiving the formolised vaccine. Moreover the absence of dead tissues diminishes the risk of local abscess.

That the small amount of antigen contained in one hundred minimal infective doses of the virus should, after inactivation, be sufficient to stimulate the creation of a high degree of immunity appears very remarkable, and this observation leads to speculation as to whether the inactivated virus may not be capable of some form of multiplication in the animal body. This possibility was briefly investigated in two ferrets which had received injections of inactivated virus and were killed eight days and eleven days later respectively. Post-mortem these animals showed no naked-eye changes and 0·1 grm. of their spleens injected into normal ferrets produced no symptoms and did not lead to the development of immunity in the injected animals. This experiment by itself is not conclusive, but it at any rate justifies the statement that there is no direct evidence that the inactivated virus is capable of multiplication in the body of the inoculated ferret.

The duration of the immunity to which the inactivated virus gives rise is a point of some importance. Sufficient time has not yet elapsed to allow a final conclusion to be drawn, but it can be stated that an inoculation of approximately 100 minimal infective doses of the virus, after inactivation, is followed by a period of resistance of at least five and a half months, the longest period tested (Table V).

The rapidity with which the immunity is developed has not been studied except in a few trial experiments in which a small dose of living virus was given at the same time as a larger dose of the inactivated virus. An attack of acute distemper resulted from these experiments and the question was not further investigated.

In order to determine whether the immunising factor is associated with the inactivated virus particles or is present in solution in the surrounding fluid, a single experiment was made with the help of Dr. Elford, who very kindly filtered some of the vaccine through a somewhat close collodion membrane (average pore size 0·12μ.) A small amount of deposit stained a dark blue remained on the membrane, the filtrate being perfectly clear and only showing a faint tinge of colour. One quarter of the deposit left on the membrane produced a solid immunity in a ferret, while half of the total ultrafiltrate failed to protect another animal. It seems clear therefore that the immunising factor is associated with the inactivated virus particles and is not in solution in the suspending fluid.

As already stated, the results obtained in ferrets have not yet been satisfactorily extended to the dog, owing to the lack of suitable susceptible animals, but in preliminary trials a solid state of immunity to the living virus has been produced in two dogs with doses of inactivated virus which are quite comparable with those used in the case of ferrets.

The question whether the method of prophylactic inoculation

described in this paper is capable of any general practical application can only be solved by its more extended trial on a larger series of animals, particularly dogs. The small dose of the vaccine, the absence of the coarser tissue elements, the quick and easy technique and the fact that the vaccine can be dried immediately after preparation and kept without any obvious diminution of its immunising properties are points at least worthy of consideration.

Conclusions.

1. The virus of canine distemper, as it exists in filtrates or other fluids devoid of living cells, is rapidly inactivated by the photodynamic action of methylene blue.

2. Ferret virus, inactivated in this way, constitutes a good vaccine for the ferret—a single dose administered subcutaneously being sufficient to immunise. The antigenic value of virus so inactivated seems to be remarkably high, since an amount not exceeding 100 minimal infective doses was sufficient, after inactivation, to give rise to a good immunity.

3. The vaccine, being prepared from the supernatant fluid of a centrifuged spleen suspension, is free from all coarser tissue elements and so is rapidly absorbed on subcutaneous injection.

4. The antigenic value of the vaccine is ultimately reduced by prolonged or excessive irradiation, but this may be safely extended to at least 24 times that necessary for inactivation without materially diminishing the antigenic value, so that the risk of accidental over-irradiation is not great.

5. Ferrets which had received a single small dose of the vaccine subcutaneously were found to be still immune after five and a half months—the longest period tested.

6. Drying the vaccine does not appear to affect its immunising properties, and dried ferret vaccine which has been kept at room temperature up to now for two months has protected ferrets satisfactorily.

7. A vaccine prepared from dog spleen was effective in protecting the dog.

8. While the virus, as it exists in filtrates or other fluids devoid of living cells, is highly susceptible to the photodynamic action of methylene blue, the virus contained in living tissue is comparatively resistant to the dye. This point is emphasised, as the accidental presence of living cells is a common source of failure to inactivate suspensions of the virus.

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THE VIRUS DISEASES OF ANIMALS WITH SPECIAL REFERENCE TO THOSE OF POULTRY*

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When preparing these notes on the virus diseases of fowls it soon became evident that it would be difficult to deal adequately with the subject if attention were confined to poultry diseases, and that it would be better to consider them in relation to other animal virus diseases.

It is only within the last ten years that any serious study has been made of avian diseases, and although considerable progress has been made our knowledge of the finer details of the virus infections is still meagre.

It is unnecessary to discuss here the nature of viruses; it will be sufficient to mention that the concensus of opinion in this country is that they are living organisms.

The virus diseases of animals are in general more virulent and spread more quickly than bacterial diseases, but there is a wide range in their degree of infectivity and their modes of dissemination.

Filtration.

Filterability does not differentiate definitely this group of diseases, as it is known that certain small bacteria can pass through bacteriological filters. The causal agents of some diseases are almost certainly viruses, yet it is difficult, if not actually impossible, to pass them through a filter which will retain visible bacteria; the viruses of fowl pox, vaccinia and most of the plant viruses are examples of this type.

Filtration technique is complicated, contains numerous pit-falls, and its success depends on many diverse factors. For example, the electrical charge of the virus and of the filter, the adsorption of the virus by the filter, the pressure, temperature and duration of filtration, are important factors to which due consideration must be given. In spite of its limitations, filtration is one of the principal means of diagnosis of virus diseases.

Cultivation of Viruses.

It is generally acknowledged that the artificial cultivation of viruses is the most promising line of enquiry into this group of diseases, and that when they can be successfully cultivated in a cell-free medium our knowledge of them will be rapidly increased.

* Two lectures (abridged) delivered at the Royal Veterinary College on February 19th and 26th, 1932.

Remarkable progress has already been made in this direction, and what appeared unattainable a few years ago is now almost a matter of routine. This progress has been mainly the result of the success achieved in the growing of living tissue outside the animal body, as it is in symbiosis with living tissues that viruses have been cultivated.

It is unnecessary to give details of the various media used, but one type has been hen-serum or plasma with embryonic tissue of chickens diluted with Tyrode solution. Vaccinia virus has been successfully cultivated in this medium. Findlay cultivated fowl pox virus in medium containing chick embryo brain and skin. Glover has recently cultivated the virus of pigeon pox using a similar kind of medium. The virus of foot and mouth disease has been cultivated by the Maitlands in medium containing guinea-pig tissues.

The successful cultivation of viruses appears to depend on the presence of living cells in the medium. Eagles and McClean claim to have cultivated vaccinia virus in a cell-free medium, but their work has not yet been confirmed.

It is of interest to recall in this connection that the virus of contagious bovine pleuro-pneumonia was first cultivated over thirty years ago in a cell-free medium.

The Dissemination of Virus Diseases.

The two principal means by which virus diseases are disseminated are by (1) affected animals, (2) infected carcases.

1. In undeveloped countries with uneducated populations, the illicit movement of animals is one of the principal ways by which contagious disease is introduced and disseminated. This source of infection is of little importance in settled countries.

2. It has been proved that the viruses of certain animal diseases are capable of remaining viable for relatively long periods in the tissues of preserved carcases, and this is undoubtedly the commonest method by which these infections are introduced and it is the most difficult to control. It is important to note that a virus may be present in the carcase of an infected animal killed during the thermal reaction, before the development of visible symptoms.

Minett has shown that the virus of foot and mouth disease can survive at least 76 days in the bone marrow of frozen beef and bacon carcases and 42 days in chilled carcases. In dry-salted bacon carcases the bone marrow was found by guinea-pig inoculation to be infective after 43 days. Further work by the British Foot and Mouth Disease Committee proved that the virus may remain viable for 80 days in the bone marrow of frozen beef carcases, and 145 days in the tendon tissue of guinea-pigs.

In experiments carried out at the Ministry's laboratory, Weybridge, it was found that swine fever virus survived 73 days (not the end-point) in the bone marrow of salted, and of chilled carcases. It survived 17 days in the skin and muscle of pigs killed *before* the appearance of clinical symptoms ; there was some evidence that the

virus was still viable in salted muscle after 73 days. The virus survived 42 days in salted skin and muscle of pigs killed *after* the appearance of clinical symptoms. It survived for 37 days (not the end-point) in bone marrow and muscle, and for 19 days in skin of smoked carcases.

Purchase has shown that the virus of fowl plague can survive 287 days in the muscle and 303 days in the bone marrow of " chilled " carcases.

In work recently carried out at Weybridge, the virus of Newcastle disease of fowls was found to survive for six months in bone marrow and muscle of " chilled " carcases. It is probable that similar results would be obtained with some of the other animal viruses.

In view of these facts it is necessary to modify our ideas and in future to regard the *preserved carcases, and not the living animal*, as the most dangerous channel by which certain virus infections can be introduced.

This source of infection probably explains the origin of some of the outbreaks of foot and mouth disease and swine fever which occur in this country, and possibly the outbreak of Newcastle disease of fowls which occurred in 1925. In America two outbreaks of foot and mouth disease have occurred during recent years and one of them was proved to have originated from infected meat.

The swine fever outbreak in New South Wales in 1927-28 was believed to have been introduced through garbage obtained from overseas ships, and there is strong circumstantial evidence that on each occasion the disease has appeared in Australia it has been introduced through either pork or bacon or in garbage from ships.

Virus Carriers.

By the term " carriers " is meant a recovered animal which harbours and transmits the virus although apparently in good health.

It is necessary to be guarded in expressing an opinion as to the occurrence of carriers in any particular virus disease, because existing methods for the detection of minute quantities of virus are inadequate and unreliable. The only practical method at present available is the inoculation of susceptible animals with some of the suspected material. There might be, however, a considerable quantity of virus distributed throughout the system, yet the minute amount present in the inoculum might be insufficient to set up infection. On the other hand, the minimum infective dose of some viruses is extremely small, *e.g.* :—

Swine fever = 1/300,000 to 1/400,000 c.c. (blood).
Rinderpest = 1/1,000 to 1/5,000 c.c. (blood).
Fowl pox = 1/1,000,000 c.c. (suspension of crusts).
Foot and mouth disease = 1/5,000,000 c.c. (vesicular fluid).

As a rule centrifuging viruses for long periods at high speeds brings about little or no concentration ; Bedson has shown that the virus of psittacosis can, however, be almost completely thrown down

by centrifuging. A method of concentration frequently employed is the adsorption of the virus on certain materials, such as animal charcoal, kieselguhr, red blood corpuscles, bacteria, etc.

Experiments of considerable interest have been reported by Olitsky and his collaborators on this problem. They proved by cataphoresis of the tissues of vaccinated rabbits, and of monkeys recovered from poliomyelitis, the existence of virus which could not be demonstrated by the direct inoculation of the same material.

Kligler was able to concentrate the virus of fowl pox by employing the same technique, and with the particular strain used he succeeded in reducing the period of incubation from nine to five days.

In the literature on virus diseases vague statements repeatedly occur which leave the impression that the existence of carriers has been proved beyond all question. It is, however, extremely doubtful if any indisputable evidence exists in support of that contention. It has not been proved by *animal inoculation* that cattle recovered from rinderpest or birds recovered from fowl pox, fowl plague, or Newcastle disease, harbour virus in the system, and circumstantial evidence based on extensive clinical experience is strongly opposed to the existence of carriers in these diseases.

According to Michalka (1931), 76 per cent. of pigs naturally infected with swine fever or immunised against it by the serum-virus method harbour the virus in the lymphatic glands for a period of ten months.

Manninger and Csontos (1932) repeated Michalka's experiments and were unable to find any evidence of virus in the lymphatic glands of immunised, artificially infected, or naturally infected pigs after an interval of twenty-six days. They suggest that Michalka's results may have been caused by accidental infection of his experimental pigs with swine fever or by an error in diagnosis. Hegyeli (1932) also failed to confirm Michalka's findings and obtained similar results to those of Manninger and Csontos. He showed in addition that there was no evidence of virus in the internal organs or lymphatic glands of immune pigs five days after the inoculation of large quantities of virus.

It would appear from recent work by Waldmann that foot and mouth virus can persist in the blood and urine of cattle recovered from artificial infection. He claims that in blood precipitated by acetone the presence of virus was demonstrated 158 days after infection, and by adsorption of the virus on charcoal it was shown to be present in urine 246 days after infection. These results have not yet been confirmed.

According to the reports of the British and American Foot and Mouth Disease Committees, no evidence of " carriers " was found among cattle recovered from natural infection.

Theiler states that horses recovered from African horse sickness are not virus carriers, or at least for only a short time. It has been observed during the preparation of horse sickness immune serum

that the elimination of virus from the system of artificially infected horses may occasionally take as long as three months. A point of interest in this connection is that a considerable percentage of recovered horses contract the disease a second time when exposed to natural infection. If these animals were virus carriers it appears reasonable to assume that they would be immune, or at least resistant, to acute infection. Theiler suggests that mosquitoes, possibly of the genus Ochlerotatus, may act both as reservoirs and as vectors.

In dog distemper the existence of virus carriers has not been proved.

It is frequently claimed that birds recovered from fowl pox retain the virus over long periods in the internal organs. This contention is probably incorrect, as even in birds exhibiting active lesions of the disease it is only occasionally that virus can be demonstrated in the internal organs.

An example of a true virus carrier is the adult guinea-pig infected with salivary (submaxillary) virus ; these adult guinea-pigs are immune to inoculation with the virus. Young guinea-pigs are susceptible to the intracerebral inoculation of the virus, but serial passages from brain to brain are unsuccessful.

Other examples of virus carriers are found among the mosaic diseases of plants. When cuttings are taken from *apparently* healthy plants and grafted on to truly healthy plants they induce mosaic disease.

It is possible that, with the elaboration of more refined technique, it may yet be shown that animals recovered from certain diseases retain minute quantities of virus over long periods in the system.

It would appear from clinical experience that in the majority of the virus diseases the recovered animal is not, after a brief lapse of time, a factor of serious importance in the dissemination of infection. If, however, the persistence of minute quantities of virus in recovered animals is eventually proved, the possibility of dissemination from this source cannot be safely ignored. There are many unknown factors in disease transmission, and there is ever present the possibility that certain animals may have an abnormally low resistance to infection, and that a minute dose of virus might be sufficient to infect them and thus afford a starting point to the disease.

Immunity.

Recovery from virus infections is generally followed by a lasting immunity ; there are exceptions, as for example, in foot and mouth disease where the duration of immunity varies from 12 to 30 months ; and herpes infection in man against which it appears doubtful if any immunity is developed.

Some observers claim that immunity is dependent on repeated re-infection, while others consider that it is the result of the persistence of virus in the system.

The sera of animals recovered from certain virus infections are weak in protective properties although the animals are actively immune and this fact has led to the belief that protection is mainly the result of a local or tissue immunity as opposed to a general or humoral immunity; examples of this type are found in vaccinia, fowl pox and foot and mouth disease.

It has been proved that anti-bodies occur in the sera of animals recovered from these diseases; viricidal properties, precipitins and complement fixing anti-bodies have been demonstrated by various workers. Ciuca succeeded in distinguishing the different types of foot and mouth virus by means of the complement fixation test. Gordon believes that agglutinins are present in the sera of animals recovered from vaccinia because " vaccine granules " are agglutinated by such sera. Ledingham has obtained the same results with the elementary bodies in fowl pox and vaccinia.

Immunisation against Virus Diseases.

The measures which should be adopted for the control of a virus disease will depend on the geographical situation of the country, the incidence of the disease and its economic importance.

On an island like Britain the slaughter of affected and in-contact animals is, when practicable, the quickest and most economical method of eradication. When, however, the incidence of a disease surpasses a certain point this method becomes economically unsound and cannot be practised.

One or other of the various methods of immunisation may then be carried out. The following are the principal methods in general use :—

(1) Dead virus.
(2) Modified virus.
(3) Live virus.
(4) Serum-alone.
(5) Serum-virus.
(6) Vaccine virus.

Dead Virus.

The majority of investigators are agreed that for the control of animal disease dead virus, whether killed by physical or chemical means, confers insufficient protection to be of practical value.

Live Virus.

In America this method is extensively used for the protection of birds against fowl pox, and it gives moderately good results. Its application should be restricted to birds under four months of age, as in adults it causes constitutional disturbance and loss of condition, and frequently gives rise to generalisation of infection and a considerable mortality may result.

The use of vaccines containing live fowl pox virus has been supplanted in most countries outside of America by those consisting of pigeon pox virus.

Vaccines containing live virus are also used against blue tongue (catarrhal fever) of sheep and contagious bovine pleuro-pneumonia.

Serum-alone Method.

The use of hyperimmune serum is theoretically sound, but it frequently fails in practice and, in my opinion, is of relatively little value for the control of animal diseases. The serum is expensive to produce and confers only a passive immunity of 10 to 20 days duration.

In those diseases to which the method is applicable immune serum confers a certain degree of protection; it does not prevent infection. In practice by the time the disease is reported and diagnosed and serum is obtained, a large proportion of animals are already infected and in these circumstances the method is of little value.

If immune serum is given to in-contact animals when the disease first appears it frequently gives excellent results, the majority of the animals acquiring an active immunity by contracting natural infection while partly immune.

The manner in which immune serum acts on virus is not fully understood, but it is known that it is not a mere union of the two with a resulting destruction of the virus.

It has been shown in fowl plague and vaccinia that a neutralised mixture of serum and virus can be reactivated by diluting the mixture (10-100). If the diluting is delayed beyond a certain time a permanent union between serum and virus takes place and the mixture cannot be reactivated.

C. H. Andrews has shown that virus may attack cells within half an hour of entering the body and that once the virus has entered the cell the immune serum can no longer affect it. When immune serum is present in the system the virus cannot attack the cells, but if the virus gets a start of the serum only that portion of it can be neutralised which has not yet entered cells.

This confirms clinical experience that immune serum is of prophylactic value, but of little curative value.

Serum-Virus Method of Active Immunisation.

It is possible that the serum-virus method of immunisation could be successfully employed against fowl plague and Newcastle disease, but the large number of fowls which would be required for the provision of serum makes the method impracticable. Cooper, working in India on Newcastle disease, attempted the preparation of immune serum on donkeys, but was unsuccessful.

Use of the serum-virus method has until recently been restricted to rinderpest, swine fever and horse sickness, and the possibility of

its employment against distemper is now being investigated. It appears to give favourable results for the immunisation of monkeys against yellow fever.

The method consists of the simultaneous injection of immune serum and live virus, usually on opposite sides of the body. The immune serum is prepared in the usual way and is standardised by titration on susceptible animals. The virulence of the virus is tested by inoculation into susceptible animals.

A point of practical importance is that in immunisation against rinderpest and swine fever it is not necessary to balance the dose of serum with virus, which means that a dose of serum sufficient to block out all reaction may be given without interfering with the subsequent active immunity ; this principle does not apply to the immunisation of young calves against rinderpest. It is especially valuable in the immunisation of cattle which are " carriers " of piroplasms or trypanosomes, as by controlling the reaction the " resuscitation " of the blood parasites can usually be avoided.

It would appear from American experience that there is little variation in the susceptibility of pigs to swine fever, so standard doses of serum, based on the weight of the animal, can be given. In cattle, however, there is a wide difference in the susceptibility of breeds and races to rinderpest.

In Egypt the dose of serum required for the protection of Cyprus cattle against rinderpest is about six times as great as that required for Egyptian cattle. And in India the dose of serum for hill cattle is 18 times greater than for plains cattle. It should be noted in this connection that mortality as a result of natural infection is not always a reliable criterion of susceptibility. In Indian hill cattle, and in imported breeds, the mortality is 98 to 100 per cent., yet imported cattle require three to four times as much serum for protection as is necessary for hill cattle. The dose of serum must, therefore, be in proportion to the susceptibility of the animals inoculated, and it must be ascertained by preliminary titration experiments.

The immunity conferred by the serum-virus method is usually regarded as persisting throughout life, but as nearly all work on this point has been carried out in countries where the particular diseases are enzootic, it has not been possible to eliminate completely the risk of re-infection taking place, and the immunity being reinforced. In experiments carried out on a Government Estate in Egypt it was shown that cattle were solidly immune to rinderpest nine years after immunisation, and as far as was known they had not been in contact with the disease during that period.

In controlled experiments on rinderpest carried out in India, Edwards showed that solid immunity lasted for two years, after which period it gradually waned. He pointed out that the duration of immunity depended probably on many factors, such as the age of the inoculated animal, the breed, the initial susceptibility to infection and the degree of reaction following inoculation.

It is surprising how frequently it is stated that animals immunised by the serum-virus method may develop into " carriers," and this objection is advanced against its use for the control of swine fever in some parts of Europe where the disease is enzootic. There seems little grounds for believing this and there is certainly no proof that inoculated animals develop into *true* carriers. Live virus having been inoculated into them, it takes some time for it to be completely neutralised or eliminated.

Dorset showed that swine fever virus was eliminated by the twenty-first day, and Edwards found that cattle inoculated against rinderpest were non-infective after the fourth day. In the case of cattle and pigs this period of infectivity is of little practical importance as all animals on infected premises are invariably inoculated at the same time, and a period of three weeks isolation after inoculation would eliminate the risk of infection being disseminated from this source.

Proof of the efficacy of the method is that every year in America over 20,000,000 pigs are immunised by it against swine fever ; and the total number inoculated now reaches hundreds of millions. In Egypt in 1923 over 250,000 cattle were immunised against rinderpest with a mortality from all causes of less than 0·1 per cent. Edwards records the immunisation of 50,000 cattle against rinderpest in Mysore State with a loss from all causes of less than 0·3 per cent.

The disadvantages of the method are that it is laborious and expensive. For immunisation against swine fever it is also essential that the pigs should be in good health. Pigs heavily infested with parasites or affected with necrotic enteritis (B. suipestifer infection) frequently do not stand up well to the inoculation and there may be some mortality. This difficulty is being overcome by the immunisation of pigs at from four to six weeks of age, that is before they become heavily infested with parasites or contract B. suipestifer infection.

There is little justification for the assertion so frequently made by European workers that the serum-virus method is dangerous ; any method of immunisation which involves the use of live virus is dangerous in the hands of untrained or inexperienced persons, or if the materials used are unreliable or the animals inoculated are in a bad state of health, but the wide application of the method is ample testimony to its efficacy and economic value.

It is inadvisable to accept without question the time-worn arguments against active immunisation against rinderpest and swine fever. In any heavily infected country where the stamping out of disease by slaughter is not an economic proposition the only alternative worth considering is active immunisation.

The combined efforts of laboratory workers in various parts of the world have succeeded in bringing this method of immunisation to a high state of efficiency, and it is in the interests of stock owners that it should be fully exploited.

Modified Virus.

It is frequently observed in the course of investigations that the inoculation of some particular sample of virus, which has become attenuated either through age or through the addition of some chemical, gives rise to only a mild reaction, yet the inoculated animal develops a strong immunity. The object of much of the experimental work on virus immunisation during recent years has been to produce constantly by artificial means this degree of attenuation.

The only method that need be considered under this head is virus inactivated or attenuated by the addition of formalin. Formalised virus has been tested during recent years against various animal disease, *e.g.* foot and mouth disease, rinderpest, rabies, dog distemper, fowl plague, fowl pox, Newcastle disease, etc.

Formalised virus confers a solid immunity against certain diseases, the duration of which varies within wide limits but is in all cases fairly short ; multiple inoculations increase the strength and duration of the immunity.

The inoculation of a formalised vaccine may produce a complete temporary immunity which will prevent any subsequent reaction on inoculation of potent live virus. The best results are likely to be obtained by the inoculation of a vaccine which will allow of some systematic reaction, however slight, on subsequent inoculation of live virus. The main difficulty lies in obtaining constantly the required degree of attenuation in the vaccine.

The method is of considerable importance for the worker in human pathology because in immunising against the diseases of man the strength and duration of the immunity must always be subordinated to safety. In the case of animal diseases the economic factor is the most important, and a method which will confer a durable immunity as the result of one inoculation, though it may cause some constitutional disturbance, or even a small mortality, is to be preferred to one that is safe but less effective. It would appear from present indications that immunisation by means of formalised vaccine *alone* is likely to remain of only academic interest to the worker in animal pathology.

Opinion is divided as to whether formalised vaccine contains dead or live virus. Some workers claim that when the virus is dead the vaccine confers no immunity and that it is only when the vaccine contains living virus that it confers protection.

If the immunising power of formalised vaccine depends on the presence of minute amounts of living (or modified) virus, the value of the method may possibly be limited because of the difficulty of obtaining constantly the required degree of attenuation in the vaccine. The virus content of different samples of material is variable, and consequently, in the absence of practical methods of standardisation, the results are likely to be inconstant.

Vaccine Virus.

A method of immunisation which appears to have considerable possibilities is the inoculation of formalised vaccine followed later by live virus. This method has been employed by Laidlaw and Dunkin for the immunisation of dogs against distemper. They now use vaccine followed by dried virus, and according to Dalling the method is highly efficient.

The vaccine-virus method should prove to be of particular value against the virus diseases of fowls, especially as the serum-virus method is impracticable on account of the difficulty of obtaining sufficient quantities of serum.

Fowl Pox.

Fowl pox is a contagious disease caused by a virus, characterised by eruptive lesions on the unfeathered parts of the head, by yellowy, cheesy membranes in the mouth and occasionally by an oculo-nasal discharge. Any one or a combination of the lesions may be present.

Epidemiology.

The common method of propagation is through wound infection and direct contact between diseased and healthy birds is usually necessary for infection to take place.

Kliger, Muckenfuss and Rivers have proved the transmission of the virus by Culex and Aedes mosquitoes. This may be an important method of transmission in warm climates, but it is improbable that mosquitoes play much part in the dissemination of infection in this country, as the disease is most prevalent during the cold season of the year when mosquitoes are scarce or absent.

It has been erroneously stated that healthy fowls do not contract infection when held in the same cage as affected birds. It is an established fact that a susceptible bird rarely, if ever, fails to become infected in cage experiments.

The Virus of Fowl Pox.

The filterability of fowl pox virus has been the subject of considerable controversy, but the virus is now generally accepted as belonging to the class of filter passers. The difficulties encountered in filtering it are probably the result of it being intracellular.

The virus is resistant and may remain viable for years at room temperature. Field experience points to it being capable of surviving for long periods, probably as long as 12 months in poultry houses and runs.

There is no evidence of the existence of immunologically distinct strains of fowl pox virus.

Inclusion Bodies.

There is a considerable literature on inclusion bodies and opinion is divided as to their nature. Some workers regard them as cell

degenerations, while others believe that the minute bodies contained within the inclusions are in reality living agents.

In fowl pox the cell inclusions are known as " Bollinger bodies " ; they are confined to the cytoplasm of the epidermal cells of the lesions ; they are constantly present and are sufficiently characteristic to be of diagnostic value.

Goodpasture and Woodruff have shown that the inoculation of a fowl with a single " Bollinger body " will give rise to typical fowl pox lesions. Several investigators have demonstrated that Bollinger bodies consist of an aggregation of minute coccoid structures, uniform in size and shape, known as Borrel bodies. A stained smear of a broken up inclusion body has been shown to contain as many as 20,000 Borrel bodies.

Ledingham has succeeded in obtaining, by means of a special technique, practically pure suspensions of the " Borrel bodies," and with these suspensions he has carried out serological tests. He found that in hanging drop preparations of the elementary bodies no clumping occurred with the sera of normal chickens, but the sera of recovered and hyperimmunised fowls clumped the bodies in dilutions up to 1 : 160.

Bedson has prepared suspensions of psittacosis inclusion bodies and has proved that these are agglutinated specifically by an anti-psittacosis serum and fix complement in its presence.

A full consideration of the existing evidence would appear to be strongly in favour of the inclusion bodies in fowl pox and psittacosis being the virus.

Distribution of Virus in the System.

During the course of the disease virus can occasionally be demonstrated in minute quantities in the blood, but its presence there is transient and is subsequent to the development of epidermal lesions. In our experience the virus can only occasionally be demonstrated in the internal organs, and infectivity of the organs appears to be dependent upon their blood content.

According to Lipschutz, pigeon pox virus invades the internal organs of pigeons and persists in them for several weeks after the skin lesions have disappeared. We have recently carried out some work on this point and were unable to find any evidence of virus in the internal organs *of recovered pigeons.*

We have been unable to find any experimental evidence of the existence of carrier birds, and this finding is strongly supported by clinical experience.

In summing up this phase of the disease, a reasonable view appears to be that fowl pox virus can occasionally be demonstrated in the internal organs of fowls exhibiting active lesions of the disease, but there is no reliable evidence that virus persists in detectable amounts in the internal organs of fowls *after* the disappearance of skin lesions.

Immunisation Against Fowl Pox by Means of Pigeon Pox Virus.

Pigeon pox was regarded until recently as being distinct from fowl pox. Pigeon pox virus is readily inocuable into fowls but infection can be only exceptionally induced in pigeons with fowl pox virus. It has been shown that by rapidly passaging fowl pox virus through pigeons a strain of virus can eventually be obtained which will " take " constantly in pigeons.

From this starting point it was proved that pigeon pox virus is immunologically indistinguishable from fowl pox virus and that the differences which exist between the viruses are merely the result of species adaptation.

Although pigeon pox virus produces lesions on fowls at the site of inoculation, it shows no tendency to cause generalised infection. Furthermore, a fowl exhibiting active pigeon pox lesions is not infective for susceptible fowls placed in contact with it.

This knowledge has been exploited in the preparation of a vaccine from pigeon pox virus for the protection of fowls against fowl pox.

The vaccine confers a solid immunity against natural infection with fowl pox. The immunity is fully established about the fourteenth day and lasts about six months.

It confers considerable, but not complete, protection against severe artificial infection with fowl pox virus. The vaccine does not give rise to any constitutional disturbance or loss of condition, or interfere with egg production.

Fowl Plague.

Fowl plague is an acute infectious disease of birds caused by a virus. It chiefly affects fowls, turkeys and geese, but many species of wild birds appear to be susceptible. *Pigeons are insusceptible.*

The incubation period under natural conditions varies from three to five days or even longer. As a general rule, the birds are found dead. The percentage mortality varies within wide limits and ranges from 40 to 100 per cent.

Diagnosis.

Diagnosis is based on the history of a rapidly-spreading disease with a high mortality, failure to isolate any visible bacteria (which eliminates fowl cholera and fowl typhoid), and the absence of respiratory symptoms (breathing through the half-opened mouth is a prominent symptom in Newcastle disease and infectious laryngotracheitis).

The inoculation of pigeons is a useful aid to diagnosis, as they are resistant to infection with fowl plague virus and are susceptible to fowl cholera and Newcastle disease.

Post-mortem Lesions.

The essential lesions in fowl plague are petechial hæmorrhages which may occur in almost any part of the body. Their principal

sites are the lining membrane of the proventriculus, the gizzard fat, the inner surface of the sternum and the pericardial sac and heart muscle.

The artificially induced disease is septicæmic and death usually occurs in from 18 to 48 hours. On autopsy of affected birds no other changes may be found than those associated with septicæmia, but as a rule petechial hæmorrhages are present in the predilection sites.

Virus.

It is generally recognised that the virus is extremely minute and can be readily passed through Berkefeld and Chamberland filters. It cannot be concentrated by centrifuging at ordinary speeds, but it has been claimed that centrifuging over prolonged periods (11 hours) at a high speed brings about some concentration.

It is extremely virulent and usually the 1/1,000,000 c.c. of blood is sufficient to kill a fowl ; the minimum lethal dose of some strains is said to be as low as the 1/1,000,000,000 c.c.

Artificial Culture of the Virus.

In 1908, Marchoux reported the successful cultivation of the virus in defibrinated fowl blood ; ten successive passages were made. Landsteiner and Berliner (1913) claim to have confirmed Marchoux's result. Todd (1928) repeated Marchoux's experiment using similar technique, but obtained no evidence of multiplication. It appears improbable that the virus has yet been successfully cultivated in a cell-free medium.

Immunisation.

Juan and Staub (1920), claim to have produced solid immunity by a single intramuscular inoculation of virulent blood (10 c.c.) heated for 45 minutes at 60° C. This method has been tried by various other workers but without success.

Staub (1926) reported good results with a formalised vaccine made from the spleens of affected fowls. This method has also failed to be confirmed by other workers. Formalised vaccine does, however, confer some degree of protection against natural infection, but apparently confers little against artificial infection with virulent blood.

Todd (1928) reported that he succeeded in immunising fowls by the use of a phenol-glycerine vaccine prepared from the livers of birds dead of the disease. It was necessary to give three doses of vaccine at intervals of a week.

Purchase (1930) repeated Todd's experiments and, in his hands, the phenol-glycerine vaccine did not give good results ; only 20 per cent. of fowls proved immune when tested seven days after the last dose of vaccine.

Fowl plague is particularly suitable for the study of virus immunity experiments as the naturally susceptible host—the fowl—is cheap and easily procurable, the virus is readily filtered, the filtrates are highly virulent and results are quickly obtained.

Newcastle Disease.

This disease may be described as an acute, infectious disease of fowls which greatly resembles fowl plague. It is caused by a virus and is characterised by a difficulty in respiration and a high rate of mortality (98-100 per cent.).

The disease has been reported from England, Dutch East Indies, Phillipine Islands, Ceylon, India and Australia. A disease said to be fowl plague has been reported from Korea, but from the description of the symptoms and from the fact that pigeons were susceptible to infection it is probable that it was an outbreak of Newcastle disease.

A satisfactory name has not yet been found for this disease, as in view of its widespread distribution the name " Newcastle disease " is obviously unsuitable, but it can be said in favour of retaining it for the present that it is more suitable than the only suggested alternative—pseudo-fowl-plague. Newcastle disease is a distinct entity and should not be so named as to create confusion with an existing disease.

Period of Incubation.

This varies from four to eleven days ; five days is about the average period when fresh virus is used.

Virus.

The causal agent is a virus which can be passed through Chamberland L3 and Berkefeld candles, and also, but with more difficulty, through Seitz filters. The disease can be passaged through a series of fowls by the inoculation of Berkefeld filtrates of mouth exudate. The mouth exudate is particularly suitable material for filtration experiments on account of its high potency and the ease with which it can be filtered. Titration experiments have shown a wide variation in the potency of different samples ; the end-points have varied between 1 : 1,000 and 1 : 2,000,000.

Symptoms.

In addition to the usual symptoms of sickness, there is a yellowish-white, watery diarrhoea with a characteristic, nauseating smell. In the majority of affected birds there is a thick mucus discharge from the nostrils and a varying amount of frothy exudate in the mouth which occasionally hangs in threads from the end of the beak.

The most characteristic symptom is noticed in connection with respiration. This is a long, gasping inhalation through the half-opened beak ; about 70 per cent. of naturally infected fowls exhibit

this symptom. A somewhat similar respiratory symptom occurs in fowls affected with laryngotracheitis.

Lesions.

There are no characteristic lesions and in many cases nothing more is found than the organic changes associated with fever. Petechial hæmorrhages are present in about 20 per cent. of cases. These occur on the gizzard fat, pericardial sac, heart muscle, lining membrane of the proventriculus and on the intestinal mucosa; hæmorrhages rarely occur on more than one of these sites in the same bird.

Differential Diagnosis.

Fowl Plague.

Newcastle disease bears considerable resemblance to fowl plague, but is distinct from it in many respects.

1. The average period of incubation of Newcastle disease in artificially infected birds is five days and the duration of visible illness varies from one to three days or longer. The frequent passage of the virus through fowls over a period of eight years has not brought about any reduction in the period of incubation.

In plague the period of incubation after artificial infection varies from eighteen hours to two days.

It should be remembered that in artificially induced plague the bird is almost invariably found dead, usually without showing any symptoms, or at most only a very brief period of dullness and inappetence. Therefore, when reference is made to the period of incubation of plague what is usually meant is the interval between infection and death, whereas in Newcastle disease the period of incubation is followed by a well defined period of sickness lasting from one to three days.

It has been proved by cross-immunity tests that fowls hyper-immunised against Newcastle virus are susceptible to either artificial or natural infection with plague virus; and conversely, birds immune to plague virus are susceptible to either artificial or natural infection with Newcastle virus.

A further point of differentiation is that pigeons, both young and adults, can be easily infected with Newcastle virus, but are resistant to infection with plague virus.

Picard in Java, Rodier in the Philippine Islands and Cooper in India have proved by serological and cross-immunity tests that the disease in those countries is identical with that in England.

The possibility of the Newcastle virus being a strain of fowl plague virus is negatived by the marked difference shown in the incubative periods and symptoms of the two diseases, and by the clear-cut results obtained in cross-immunity and serological tests. While the existence of immunologically distinct strains of the same virus is known to occur, as, for example, in foot and mouth disease

and in vesicular stomatitis, the affections induced by the different strains are clinically indistinguishable.

It is not exceptional to find two disease entities showing many similarities, sometimes indeed so close is the resemblance that differential diagnosis is difficult. As example of this can be seen in foot and mouth disease and vesicular stomatitis ; these two diseases in cattle cannot be differentiated with certainty by clinical examination ; in pigs the mouth lesions are clinically indistinguishable.

Control of the Disease.

In view of the infectious nature of Newcastle disease and the high rate of mortality caused by it, it would be inadvisable in this country to practise any method of immunisation.

The stoppage of movements of fowls in a prescribed area and the slaughter of all fowls on infected premises are the logical methods of control.

Laryngotracheitis (or Infectious Bronchitis).

This disease appears to be at present confined to the North American continent. It is of considerable economic importance as it has caused heavy losses during recent years.

The first definite report of its existence appears to be that of May and Tittsler in 1925. They recognised the disease as being distinct from ordinary bronchitis and showed that it could be transmitted to healthy fowls by the intratracheal inoculation of exudate from the trachea of affected birds.

Fowls of all ages are susceptible ; the period of greatest susceptibility appears to be between the fourth and eighteenth months of age. The period of incubation following natural infection is from seven to twelve days.

In most outbreaks the disease appears suddenly and spreads rapidly through the flock ; 50 to 90 per cent. of fowls become affected. The height of the infection is usually reached by the seventh or eighth day, after which it rapidly subsides and in most instances has disappeared within 21 days.

Causal Agent.

All the evidence points to the causal agent being a virus. Beach set up the disease by the intratracheal, intraperitoneal and intravenous inoculation of Seitz filtrates.

Symptoms.

Affected birds exhibit a characteristic respiratory symptom during which the head is elevated, the neck extended and the air drawn in through the half-opened beak with a loud wheezing sound. At expiration the head is lowered, sometimes until the beak rests against the chest and violent coughing frequently occurs during which masses of clotted blood and mucus may be expelled.

The rate of mortality varies in different outbreaks from 5 to 90 per cent., with an average of about 20 per cent.

Post-mortem.

Except when there are complications, pathological changes do not occur outside of the respiratory tract, where they are confined to the larynx and trachea. Varying degrees of inflammation occur in the trachea, the superior and inferior larynx, and occasionally in the bronchi. In the trachea there is usually an accumulation of blood-stained, muco-purulent exudate, which in the later stages becomes caseated and deposited over the surface of the mucous membrane, and often extends into the inferior larynx and bronchi. In advanced stages this exudate may collect around the laryngeal opening and cause death by asphyxiation.

Carriers.

Komarov and Beaudette (1932) have shown that artificially-infected fowls may carry the virus in the respiratory tract for 52 days after recovery.

Immunisation.

Attempts to produce immunity with virus attenuated by the methods in general use have been unsuccessful.

"STRUCK." ENTERITIS AND PERITONITIS OF SHEEP CAUSED BY A BACTERIAL TOXIN DERIVED FROM THE ALIMENTARY CANAL. PAPER 2.

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From investigations conducted on the Romney Marsh, in the spring of 1930, the impression formed was that the number of sheep dying from what the layman calls " struck " was very considerable, and investigations showed that in 75 per cent. of the cases examined the sheep had died from a specific disease, for which the name " struck " was retained. It was attributed to a *B. paludis* toxæmia, the toxin being derived from the alimentary canal and generally, but not necessarily, associated with an invasion of the body tissues by *B. paludis*. (McEwen and Roberts, 1931.)

In 1931 and 1932 it was hoped that field investigations would extend the knowledge of this disease, and during the spring intimate contact was maintained with a number of farms on the Romney Marsh, carrying between 4,000 and 5,000 head of sheep, approximately 2,000 of which had been inoculated at the end of each winter with *B. paludis* anatoxin. It is believed that the majority of the deaths among these flocks regarded by the shepherds as cases of " struck " were reported and post-mortem examinations made. Nevertheless, only 63 suspected cases of " struck " were examined and in only eight was there evidence of the disease associated with *B. paludis*, and from seven of these cases *B. paludis* was isolated.

In both 1931 and 1932 the investigations showed that during that part of the year regarded as the " struck " season the mortality from all diseases considered by the layman as cases of " struck " was not high and that the actual losses from disease associated with *B. paludis* toxæmia or infection was small. There is no doubt that in the 1930 season the losses were very much greater than in either 1931 or 1932, and it is probable that when excessive mortality occurs it is due to the disease associated with a *B. paludis* toxæmia or infection. The evidence accumulated does not cause suspicion of the existence of any other infectious disease.

The Experimental Production of Struck.

In 1930 the disease was produced in sheep by feeding large quantities of broth cultures of *B. paludis*, three litres or more. These large quantities were objectionable, and later further experiments were undertaken in an endeavour to reproduce the

disease by feeding smaller quantities of culture. Feeding experiments were also made with laboratory animals. The disease has been produced in sheep by feeding comparatively small quantities of the bacteria, but attempts to produce disease in guinea-pigs and rabbits by feeding relatively large quantities of the bacteria gave negative results, and attempts to increase the susceptibility of the rabbit to an alimentary infection by variations in diet, *e.g.*, by feeding rabbits upon a diet of lettuce, oats, tapioca, watery extract of liver and peptone solutions, met with no success.

Sheep Feeding Experiments.

(1) An experiment was commenced to test the effect of the repeated administration of small quantities of the bacteria by dosing a number of sheep at short intervals, every second or third day, with small quantities of broth culture of *B. paludis*. This was to be carried on for several weeks, or until some of the sheep died from " struck."

Sixteen sheep at pasture were each given 50 c.c. of broth culture. The animals were dosed in the standing position with the head held horizontally. The culture was administered with great care from a small narrow-necked bottle. No coughing occurred at or soon after the administration of the culture. The following day five of these sheep were found dead and another in a moribund condition was slaughtered. Post-mortem examinations revealed most extensive lesions in the thoracic cavities of all six sheep. The pleural cavity was filled with transudate and gelatinous material, the latter being adherent in places to the lungs, pericardium and parietal pleura. The lungs were œdematous and frothy and areas of these tissues were dark red in colour. Smears made from the cut surface of the lungs showed very large numbers of *B. paludis*. In one sheep the peritoneal cavity contained 500 c.c. of fluid, and the mucosa of the ileum was hyperæmic. The remainder of the cadavers showed no lesions except those in the thoracic cavity.

The six sheep had all suffered from a gas gangrene of the lungs caused through the inhalation of some of the *B. paludis* culture.

The eleven other sheep remained well, but the experiment was not continued.

In the experiments that followed the sheep were fed with quantities of the bacteria collected from the deposits obtained by centrifuging broth cultures of *B. paludis*.

(2) Twelve sheep on pasture were each fed a gelatin capsule of between 1 and 2 c.c. capacity containing the equivalent of the bacteria present in 250 c.c. of broth culture. On the following day three or these sheep were found dead and three more were ill, and by the fourth day eight of the sheep had died. This result had not been anticipated in view of the past difficulty in

setting up alimentary infection except when several litres of culture were fed, and it was not possible to conduct post-mortem examinations until the fourth day, when, despite cold weather, putrefactive changes were advanced in most of the cadavers. One cadaver was, however, in a fresh state and presented the lesions considered typical for a sheep dying from the disease in the field, *viz.*, a copious transudation in the peritoneal cavity, marked injection of the peritoneal vessels, a congested appearance of the small intestine, and moderately congested and œdematous mesenteric lymphatic glands. Cultures from the spleen of this animal remained sterile, and smears from the mesenteric lymphatic glands and other tissues showed no micro-organisms.

Another case where decomposition changes were not advanced presented similar lesions, except that the mesenteric lymph glands were normal in appearance. Smears made from these glands and from the spleen showed bacteria morphologically similar to *B. paludis*.

The remainder of the cadavers were very decomposed, and only four were examined in detail. The lesions in the internal organs were masked by putrefactive changes. The musculature of two of the four cases presented the appearance brought about by the post-mortem multiplication of *B. paludis* and considered indicative of infection with "struck," namely a soft consistence, a dull red colour, and a partly autolysed appearance. Smears from these muscles showed very great numbers of bacteria morphologically identical with *B. paludis*. In the remaining two cases the subcutaneous and muscular tissues presented appearances compatible with the usual putrefactive process only, and smears from the muscles showed no bacteria similar in morphology to *B. paludis*.

This experiment demonstrated that disease could be preduced by administering bacteria collected and contained in a gelatin capsule when the number was at least ten times less than that which had previously been found necessary to produce disease when the bacteria were fed in broth culture.

(3) In a further experiment twelve sheep were each fed with the bacteria collected by centrifuging and obtaining the deposit from 100 c.c. of broth culture of *B. paludis*. The pasty mass was placed on the tongue with a spatula. All of the animals remained well, and this method of feeding was not carried further.

(4) The effect of administering smaller quantities of bacteria contained in gelatin capsules was next tested on five sheep. Each animal was given a capsule containing the bacteria collected from 100 c.c. of broth culture. All of the sheep remained well.

(5) A further experiment was designed to test the effect of repeated small quantities of bacteria administered by the mouth and contained in gelatin capsules. Twenty-two sheep on pastures

were used. Seven of these sheep had been inoculated two months previously each with 10 c.c. of *B. paludis* anatoxin. These animals and seven unvaccinated sheep were each fed with a capsule containing the bacilli equivalent to those present in 20 c.c. of broth culture. The remainder of the 22 animals, eight in number, were left as controls.

The day following the administration of the first capsule (second day) one of the non-vaccinated fed animals was not grazing and on the morning of the third day it died. The post-mortem examination showed a typical case of "struck," and bacilli morphologically similar to *B. paludis* and in apparently pure culture were found in smears from the peritoneal fluid, mesenteric lymph glands, liver and spleen tissue.

On the third day the remainder of the fed animals were again given a gelatin capsule containing the same quantity of bacteria. On the morning of the fifth day a fed unvaccinated animal was found dead. This animal had not been noticed to be ill on the previous day. The post-mortem examination showed a typical case of "struck" except that no microscopic lesions were found in the alimentary canal. Smears made from the kidney and spleen tissues showed no bacilli and in smears from liver tissue a few large putrefactive bacilli were seen, but smears from the mesenteric lymph glands showed numerous bacilli morphologically similar to *B. paludis*. The experimental feedings were not repeated as it was considered that the disease had been produced in two cases. The sheep were kept under observation for some weeks during which time they remained well.

All the above experiments were conducted on sheep grazing at pasture during the late winter and early spring, and in the following experiment the sheep were stall fed. Stall feeding commenced one week before the experimental feeding of culture began.

6. (*a*) Two sheep were fed on a ration of mangles and hay *ad lib.*

(*b*) Two sheep were fed on a ration of 2 lb. of maize and tapioca, equal parts, and a very small quantity of hay.

(*c*) Two sheep were each fed 2 lbs. of crushed oats and a very small ration of hay.

(*d*) Two sheep were each fed 2 lbs. of ground nut and cake and a very small ration of hay.

(*e*) Two sheep subsisting on pasture were also included.

Each of the above animals was fed a capsule containing the bacteria equivalent to those contained in 20 c.c. of broth culture. Three days later the animals were again fed with a capsule containing bacteria. Two days after the administration of the second dose of culture one of the sheep fed on oats and hay and one sheep fed on ground nut and cake and hay were found dead and

in both instances the post-mortem examination showed the lesions associated with " struck " and bacteriological examination demonstrated *B. paludis* in the tissues of these sheep.

The remaining sheep were each fed another capsule but remained well and the experiment was not continued. The experiment, however, demonstrated that stall fed sheep without access to pasture and without green succulent food were susceptible to disease produced by the oral administration of the bacilli.

The complex composition of the pastures and of diets suitable for adult sheep militated against the results of experiments with a limited number of animals giving a reliable index of any increased susceptibility brought about by different kinds of diet, but it was hoped that if a decided or marked difference in the nature of the diet and the resulting products of digestion influenced the growth of *B. paludis* in the alimentary canal and the susceptibility to disease, these might be indicated by feeding lambs upon widely different types of diets.

Preliminary feeding experiments had shown that lambs remained healthy when fed over a period of several weeks on rations of equal parts cows' milk and 17 per cent. lactose solution plus mineral mixture, or equal parts of cow's milk and 17 per cent. solution of casein plus mineral mixture. Furthermore, the animals could be maintained for several days in apparent health when the milk was replaced by either lactose or casein solution, rations of pure carbohydrate and pure protein respectively being fed. However, after remaining on these diets for six days the animals subsisting on pure carbohydrate commenced to suffer from diarrhœa and to become sickly.

Three one week old lambs were fed as follows: Lamb 1 received a ration of cow's milk; Lamb 2 received equal quantities of cow's milk and a 17 per cent. solution of lactose, and Lamb 3 received equal parts of cow's milk and a 17 per cent. solution of casein. The lambs were fed five times a day, and after they had been on the ration for one week 5 c.c. of broth culture of *B. paludis* was added to the ration at each meal for a period of six days. The animals remained healthy, and the milk in the dietaries of Nos. 2 and 3 was then replaced by lactose and casein solutions respectively and the amount of culture fed per meal increased to 20 c.c. The culture was fed for two days and then discontinued. The animals remained on these special diets for another two days. Thereafter they were placed on a normal diet. Throughout the experiment the lambs remained well.

Failure to infect adult sheep with anything but very large quantities of culture fed by the stomach tube may be due to the material entering the rumen, but in lambs the food material passes direct to the abomasum and it was surprising that the experimental lambs should have shown no signs of illness from the ingestion of relatively large quantities of culture. The failure

to infect the lambs is, however, in keeping with the epizootiological evidence, the disease, as far as is known, not occurring in lambs on the Romney Marsh.

Immunisation Experiments.

Preliminary immunisation experiments were conducted with guinea-pigs and rabbits, the animals being inoculated with anatoxin.

The anatoxin was prepared from liver broth cultures of *B. paludis* grown under a vaseline seal. The liver broth was adjusted to a *p*H of appromimately 7·6. The cultures were incubated overnight, in the morning they were cleared by centrifuging and the supernatant fluid filtered through Seitz filters. The filtrate was tested for toxicity and 0·04 c.c. inoculated intravenously into rabbits killed the animals in 15 minutes. The toxic filtrate was tested for sterility by inoculating a quantity into minced meat medium. Sufficient formalin was added to the remainder of the filtrate to give a concentration of 0·5 per cent. and this filtrate incubated at 37° C. for a period of ten days. The toxicity of the filtrate was then tested and if 5 c.c. inoculated intravenously into the rabbit produced no symptoms or illness the detoxicated filtrate or anatoxin was considered ready for use.

A number of guinea-pigs received two inoculations at 14 day intervals of 2 c.c. of anatoxin, and after an appropriate interval these animals and control guinea-pigs were inoculated with small graduated quantities of *B. paludis* broth culture. Both groups behaved with considerable irregularity, some resisting infection, while others succumbed, and as it appeared that accurate information would not be obtained from these methods guinea-pig experiments were not carried further.

Six normal rabbits were bled and their sera tested for antitoxin by inoculating mice intravenously with the mixture of toxin and serum. By the methods employed no antitoxin was detected in the sera of these animals. Three of the rabbits then received a subcutaneous inoculation of a small amount of anatoxin, and three received a larger inoculation. Nineteen days later the animals received a second inoculation of anatoxin. At intervals after both the first and second inoculations the sera of these animals were tested for antitoxin, and finally the resistance of the rabbits to the intravenous inoculation of the toxin was tested.

The toxin used in these antitoxin titration and immunity tests was the dried material obtained from the ammonium sulphate precipitate of filtrate of broth culture of *B. paludis*.

Details of this experiment are given in Table I and show that, as the result of the inoculation of anatoxin, antitoxin appeared in the sera of all the animals, but, on the whole, the rabbits receiving the larger dose of anatoxin showed the greater amount of antitoxin in their sera.

TABLE I.

Rabbit No.	*Serum Tested before Inoculation of Anatoxin.* *Standard Toxin.*				*Amount of Anatoxin. 1st. Inoc.*	*Serum Tested Seventeen Days after First Inoculation of Anatoxin.* *Standard Toxin.*			
	Dilution.	*Amount.*	*Serum.*	*Mice Inoculated iv.*		*Dilution.*	*Amount.*	*Serum*	*Mice Inoculated iv.*
1	1 : 100	0·2 c.c.	0·2 c.c.	+, +, 3-4 hrs.	1 c.c.	1 : 100	0·2 c.c.	0·2 c.c.	+, 3-4 hrs.
	1 : 50	„	„	+, +, 5 mins.		1 : 10	0·1 c.c.	„	L.
						1 : 10	0·2 c.c.	„	+, 15 mins.
									+, 5 mins.
2	1 : 100	„	„	+, +, 1 hr. 15 mins.	1 c.c.	1 : 100	0·2 c.c.	„	L.L.
	1 : 50	„	„	+, +, 15 mins.		1 : 10	0·1 c.c.	„	+, 2 hrs.
						1 : 10	0·2 c.c.	„	+, 5 mins.
3	1 : 100	„	„	+, +, 1 hr. 15 mins.	1 c.c.	1 : 100	„	„	L.L.
	1 : 50	„	„	+, +, 5 mins.		1 : 10	0·1 c.c.	„	+, 25 mins.
						1 : 10	0·2 c.c.	„	+, 3 mins.
4	1 : 100	„	„	+, 1 hr. 10 mins.		1 : 100	„	„	L.L.
	1 : 100	„	„	+, 5 mins.	10 c.c.	1 : 10	0·1 c.c.	„	L.
	1 : 50	„	„	+, +, 5 mins.		1 : 10	0·2 c.c.	„	L.
						Not diluted	0·1 c.c.	„	+, 5 mins.
5	1 : 50	„	„	+, 2 mins.	10 c.c.	1 : 100	0·2 c.c.	„	L.L.
	1 : 50	„	„	+, 5 mins.		1 : 10	0·1 c.c.	„	L.
						1 : 10	0·2 c.c.	„	L.
						Not diluted	0·1 c.c.	„	+, 1 hr. 30 mins.
6	1 : 100	„	„	+, 20 mins.		1 : 10	„	„	L.
	1 : 100	„	„	+, 4-5 hrs.	10 c.c.	1 : 10	0·2 c.c.	„	L.
	1 : 50	„	„	+, 2 mins.		Not diluted	0·1 c.c.	„	L.L.
	1 : 50	„	„	+, 5 mins.		„	0·2 c.c.	„	L.

TABLE I (*continued*).

Rabbit No.	*Amount of Anatoxin 2nd. Inoc. 19 Days after 1st.*	*Serum Tested 20 Days after Second Inoculation of Anatoxin. Standard Toxin.*				*Intravenous Inoculation of Rabbits with Toxin. 0·4 c.c. of Toxin lethal for normal Rabbits in 15 minutes.*		
		Dilution.	*Amount.*	*Serum.*	*Mice innoc. iv.*	*Time.*	*Amount Toxin.*	*Symptoms, Etc.*
1	2 c.c.	1 : 10	0·2 c.c.	0·2 c.c.	L.	0 mins.	0·4 c.c.	Nil.
		Not diluted	0·1 c.c.	„	+, 15 mins.	5 mins.	„	„
						10 mins.	0·8 c.c.	„
						10 mins.	1·6 c.c.	Slight symptoms recovered.
2	2 c.c.	1 : 10	0·2 c.c.	„	L.	0 mins.	„	Slight symptoms, recovery in 15 mins.
		Not diluted	0·1 c.c.	„	+, 2 mins.	20 mins.	„	Symptoms in 10 mins. + in half-an-hour.
3	2 c.c.	1 : 10	0·2 c.c.	„	+, 1 hr.	0 mins.	0·8 c.c.	Symptoms in 8 mins. + in 20 mins.
4	10 c.c.	Not diluted	0·1 c.c.	„	+, 4 hrs.	0 mins.	1·6 c.c.	Nil.
			0·2 c.c.	„	+, 5 mins.	15 mins.	„	Symptoms in 5 mins. + in 10 mins.
5	10 c.c.	Not diluted	0·1 c.c.	„	L.	0 mins.	3·2 c.c.	Nil.
		„	0·2 c.c.	„	+ following morning	15 mins.	„	„
6	10 c.c.	„	0·1 c.c.	„	L.	0 mins.	6·4 c.c.	Nil.
		„	0·2 c.c.	„	L.			
		„	0·4 c.c.	„	L.			
		Double strength.	0·4 c.c.	„	+, 5 mins.			

Serum and toxin mixtures were allowed to stand 1 hour at room temperature before these were inoculated into the mice.

As the degree of immunity produced by the inoculation of the anatoxin appeared to bear some relationship to the quantity of the anatoxin inoculated, it was of interest to ascertain whether concentrated dried toxin dissolved in comparatively small quantities of saline, filtered and treated with formalin, would possess a definite antigenic value. If a definite value were demonstrated it would be possible to immunise animals by the inoculation of a comparatively small volume of the material.

Dried toxin was diluted in saline and filtered through a Berkefeld candle. The filtrate was ten times as toxic as the filtrates of broth culture used in the preparation of the ordinary anatoxin. The concentrated filtrate of toxin was detoxicated by formalin in the usual manner. Three rabbits with no demonstrable antitoxin in their sera were each inoculated subcutaneously with 1 c.c.

Fourteen days later their sera were tested for antitoxin. None was demonstrated in the sera of two of the animals but antitoxin was present in the serum of the third rabbit. The animals received a second inoculation of 1 c.c. and 18 days later their sera were tested.

TABLE II.

RABBITS INOCULATED WITH ANATOXIN PREPARED FROM CONCENTRATED TOXIN. TWO INOCULATIONS OF 1 C.C. WERE GIVEN.

Rabbit.	*Serum Tested* 14 *Days after First Inoculation.* *Standard Toxin.*			
	Dilution.	*Amount.*	*Serum.*	*Mice.*
10	1 : 50	0·2 c.c.	0·2 c.c.	+, +, in 5 hrs.
	1 : 10	„	„	+, 5 mins.
				+, 11 mins.
11	1 : 50	0·2 c.c.	0·2 c.c.	+, +, few hours.
	1 : 10	„	„	+, +, 2 mins.
12	1 : 50	0·2 c.c.	0·2 c.c.	L.L.
	1 : 10	„	„	L.L.

Rabbit.	*Serum Tested* 18 *Days after Second Inoculation.* *Standard Toxin.*			
	Dilution.	*Amount.*	*Serum.*	*Mice.*
10	1 : 10	0·2 c.c.	0·2 c.c.	L.L.
	Not diluted	„	„	+, +, 3 mins.
11	1 : 10	0·2 c.c.	0·2 c.c.	+, 5 mins.
				+, few hours.
12	Not diluted	0·2 c.c.	0·2 c.c.	L.
	„	„	„	+, few hours.

The results of these tests are shown in Table II, and it will be seen that in two of the rabbits sera very appreciable amounts of antitoxin were present.

Immunisation of Sheep.

In the following experiments all the sheep were bled before they were inoculated or used as control animals and their sera was tested for antitoxin by methods similar to those employed in testing rabbit sera. In no instance was detectable antitoxin found in the sera of sheep before immunisation.

For the purpose of description the unit of toxin is regarded as the amount of toxin required to kill mice within 15 minutes of intravenous inoculation. The volume of toxin inoculated was maintained at 0·2 c.c., concentration only being the variable factor. The volume of serum inoculated was also kept constant namely 0·2 c.c. The antitoxin units mentioned below refer to the number of units per c.c. of serum and where 0·2 c.c. of serum completely neutralised a quantity of toxin lethal for mice within 15 minutes of intravenous inoculation the serum is assumed to contain at least five units of antitoxin. Similarly, had it completely neutralised five lethal doses of toxin the antitoxin content of the serum would be placed at 25 units. When complete neutralisation of toxin did not occur but the test animals survived for some hours this is taken as indicating the presence of some antitoxin but insufficient to be expressed in terms of units.

The anatoxin used for inoculation of sheep 1 to 4 inclusive was the same as that used for the immunisation of guinea-pigs and rabbits No. 1 to 6 inclusive. The anatoxin used in the remainder of the sheep immunisation experiments, except where otherwise stated, was prepared in a comparable manner to that previously described but the toxicity of the broth culture filtrates was tested on mice and 0·004 c.c. was lethal for these animals within 15 minutes of intravenous inoculation. After the addition of formalin and incubation at 37° C., 0·5 c.c. of the anatoxin was harmless for mice on intravenous inoculation.

Experiment 1.—Two sheep, Nos. 1 and 2, were each inoculated subcutaneously with 1 c.c. of anatoxin. Eighteen days later the serum of these animals was tested for antitoxin but none was demonstrable. On the same day they received a second inoculation of anatoxin, and on the seventh and again on the seventeenth days after the second inoculation their sera were tested, but in neither instance was any antitoxin found. The experiment was not carried further with these animals.

The sheep Nos. 2 and 4 were inoculated at the same intervals as sheep Nos. 1 and 2, but with 10 c.c. of anatoxin. Following upon the first inoculation no appreciable quantity of antitoxin was found in their sera, but after the second inoculation antitoxin was present in the serum of sheep No. 4, 1 c.c. of serum containing 25 units of antitoxin. The

serum of sheep No. 3, however, contained but little antitoxin, insufficient to be expressed in terms of units.

These two sheep received a further inoculation of anatoxin three weeks after the second inoculation had been given, but in neither instance did the third inoculation materially affect the antitoxin content of their sera. Four months after the final inoculation the immunity of sheep Nos. 3 and 4 was tested by feeding the sheep each with 3½ litres of broth culture of *B. paludis*. A control sheep, No. 5, was fed with the same amount of culture. The control sheep was found dead 20 hours later and the post-mortem examination revealed the typical picture of "struck." Twenty-six hours after feeding, sheep No. 3 was acutely ill and unable to stand. It was therefore chloroformed and a post-mortem examination made, and the characteristic lesions of " struck " were found. Sheep No. 4, showing antitoxin in its serum, remained well.

Experiment 2.—The effect of inoculating sheep with larger quantities of anatoxin was then tested, and the sheep Nos. 6 and 7 each received two inoculations of 50 c.c. of anatoxin at an interval of 15 days. Before the second inoculation was given the sheep were bled and their sera tested. The sera from sheep Nos. 6 and 7 both contained at least 25 units of antitoxin per c.c. Fifteen days after the second inoculation their sera were again tested and there appeared to be some slight increase in the amount of antitoxin present. The immunity to *B. paludis* culture administered by stomach tube was then tested. Sheep No. 6 and a control sheep, No. 8, were fed each with 3½ litres of broth culture. The following morning the control sheep was dull and apathetic and 24 hours after the administration of the culture it died. The post-mortem examination revealed a typical case of " struck." Sheep No. 6 remained well. Two days after sheep Nos. 6 and 8 had been fed, the vaccinated sheep No. 7 and a control No. 9 were each fed 3½ litres of broth culture of *B. paludis*, comparable in every respect to the culture fed to Nos. 6 and 8. Sheep No. 7 remained well, but the control sheep No. 9 was found dead the morning following the administration of culture. The post-mortem examination of sheep No. 9 did not show the characteristic lesion of " struck." The tissues of the head and face were œdematous and the mucous membrane of the interalveolar space showed surface erosion probably caused by the mouth gag when the culture was administered by stomach tube. Smears made from the œdematous fluid in this neighbourhood showed numerous micrococci. There was no excess of fluid in the peritoneal cavity nor was there injection of the peritoneal vessels. In the small intestine the Peyer's patches appeared congested and one of the mediastinal lymphatic glands was congested and œdematous. No other lesions were found. Pure cultures of *B. paludis* were isolated from a mediastinal lymphatic gland and from the spleen. The death of this sheep cannot be attributed to an infection with *B. paludis*, though it is probable that the bacteria found in the body represented an ante-mortem invasion and that had there been no localised infection of the tissues of the face and head the animal would have succumbed to a *B. paludis* infection of alimentary origin.

The evidence from the experiments, however, indicated that vaccinated animals with anatoxin in their serum withstood the

feeding of culture which was generally lethal for non-vaccinated animals or for animals without antitoxin in their serum.

Experiment 3.—The effect of the inoculation of 10 c.c. anatoxin was again tried.

Two sheep, Nos. 10 and 11, were inoculated each with 10 c.c. and 15 days later they were bled and their sera tested. In neither instance was there an appreciable quantity of antitoxin in the sera. The sheep then received a second inoculation of 10 c.c. anatoxin and 15 days later their sera were obtained and tested. The serum from sheep No. 10 showed no antitoxin but the sample from sheep No. 11 showed at least 25 units per c.c. It was decided to give sheep No. 10 a third inoculation of anatoxin, but by mistake this sheep was inoculated with anaculture. No increase in the amount of antitoxin present in the serum resulted and the animal was dismissed from experiment.

Sheep No. 11 and a control, No. 12, were each fed with 3½ litres of broth culture of *B. paludis*. The vaccinated sheep remained well but the control sheep No. 12 died between 24 and 32 hours after being fed. The post-mortem and bacteriological findings in this case were typical of those for a " struck " sheep examined several hours after death. This experiment further confirmed the opinion that vaccinated animals with antitoxin in their sera withstood the oral administration of culture of *B. paludis* which was lethal for animals whose serum contained no antitoxin.

Experiment 4.—The immunising value of anaculture was next tested. The original toxicity of the culture was the same as that of the filtrate from which anatoxin was prepared. The culture was rich in bacteria and its opacity was the same as that of a suspension of *B. coli* containing 5,000,000,000 bacteria per cubic centimeter. 0·5 per cent. formalin was added to the culture and the culture was incubated 10 days, by which time no toxin was demonstrable on the intravenous inoculation of 0·5 c.c. into mice, and sterility tests showed that the bacteria were no longer viable.

Two sheep, Nos. 13 and 14, were each inoculated at 16-day intervals with 50 c.c. of anaculture. Just before the second inoculation was made the sheep were bled and their sera tested for antitoxin. In both sera 25 units of antitoxin were demonstrated. Twenty-six days after the second inoculation the sera were tested but no appreciable increase in antitoxin was found. Five months after the second inoculation the sera were again tested and in the case of sheep No. 14 there was a decline in the amount of antitoxin, only five units being demonstrated.

The immunity of these sheep was now tested, in the case of sheep No. 13 by feeding it with culture and of sheep No. 14 by the intramuscular inoculation of *B. paludis* culture.

Sheep No. 13 and a control sheep, No. 15, were each fed 3½ litres of broth culture of *B. paludis*. Sheep No. 13 remained well, but sheep No. 15 was found dead 24 hours later, and the post-mortem examination showed the typical " struck " lesions.

Six days after it had received the feed of culture No. 13, along with No. 14 and a control animal, No. 16, were each inoculated intramuscularly in the thigh with 0·5 c.c. of a 1 : 8 saline dilution of broth culture of *B. paludis*. Sheep Nos. 14 and 16 were found dead the following morning. The post-mortem examinations of these two sheep

showed in each case extensive gas gangrene lesions of the inoculated leg caused by *B. paludis*. Sheep No. 13 was lame for several days but it recovered completely. The experiment again demonstrated the resistance of sheep whose serum was relatively rich in antitoxin and indicated that such animals withstood the severe test of the intramuscular inoculation of culture, whereas sheep vaccinated with anaculture but showing little antitoxin in their serum succumbed.

The anaculture did not appear to be superior to anatoxin for the immunisation of sheep.

Experiment 5.—Four sheep were included in a further experiment, both anatoxin and anaculture being used and the amount of the inoculation in each instance reduced to 5 c.c.

Sheep Nos. 17 and 18 were inoculated each with 5 c.c. anatoxin. Eighteen days later 5 units of antitoxin per c.c. of serum were demonstrated for sheep No. 17 and 25 units per c.c. of serum for sheep No. 18.

Sheep Nos. 19 and 20 were each inoculated with 5 c.c. of anaculture. Eighteen days later the sera from these animals was tested and each was shown to contain but little antitoxin—less than five units per c.c.

The immunity of sheep Nos. 18 and 20 was then tested by the intramuscular inoculation of 0·25 c.c. of broth culture of *B. paludis*. A control sheep, No. 21, was also inoculated. The control sheep died in 15 hours. Sheep No. 20 died a few hours later. Sheep No. 18 survived. Post-mortem examinations of Nos. 20 and 21 showed extensive gas gangrene lesions of the inoculated leg due to a *B. paludis* infection.

The immunity of sheep Nos. 17 and 19 was tested by feeding each with three litres of broth culture. A control, No. 22, was also fed with three litres of the culture. Eighteen hours later the control sheep died. Nos. 17 and 19 remained well. The post-mortem examination of sheep No. 22 showed a typical case of " struck."

In this experiment no superiority was shown by anaculture as an immunising agent. Again resistance to infection is seen to be linked to the presence of antitoxin in the serum. The experiment is interesting in that it shows that considerable resistance was produced in at least two of the sheep, Nos. 17 and 18, by the inoculation of only 5 c.c. of anatoxin.

Anatoxin is not considered to possess any antigenic superiority over anaculture, but it is considered preferable for use in field vaccinations on the Romney Marsh because the former is more certain to be sterile than the latter, and it is desirable to use a vaccine of assured sterility so that the vaccine itself may be exonerated from blame should gas gangrene result from its careless use. (Roberts and McEwen, 1932.)

The preparation of large quantities of culture filtrate for the production of anatoxin is tedious because of the preliminary centrifuging required to clear the culture before filtration can be satisfactorily carried out. Were filtered anaculture to prove as efficient an antigen as the ordinary anatoxin this would afford an easier method of preparing vaccine for use in the field, because upon standing the bacteria in the anaculture settle out, leaving a

clear supernatant fluid which may be syphoned off and filtered without preliminary centrifuging.

Filtered anaculture was, therefore, prepared from the same batch of anaculture used in the previous experiments.

The sheep Nos. 23 and 24 were each inoculated subcutaneously with 10 c.c. Eighteen days after inoculation their sera were tested and no antitoxin found. A second inoculation of 10 c.c. was given and 14 days later the sera tested. Sheep No. 23 showed 5 units of antitoxin per c.c. Three months after the second inoculation the sera of these animals were again tested but in neither case was antitoxin detected in a preciable quantity.

Sheep No. 23 was then fed 3½ litres of broth culture and 24 hours later it was found dead. The post-mortem examination showed the characteristic lesions of " struck."

Sheep No. 24 was inoculated intramuscularly with 0·5 c.c. of a 1 :40 saline dilution of broth culture of *B. paludis* and died from an acute gas gangrene infection. At the same time sheep No. 4 which had previously resisted feeding with 3½ litres of *B. paludis* broth culture and a control sheep No. 26 were also inoculated intramuscularly with the same quantity of the culture. Sheep No. 4 remained well, but the control sheep died of acute gas gangrene infection.

From the results of these experiments it was not considered advisable to continue further work with filtered anaculture.

As the inoculation of rabbits with concentrated anatoxin had given encouraging results, the effect of the inoculation of concentrated anatoxin was tested on two sheep. Two sheep, Nos. 27 and 28, each received two inoculations of 1 c.c. of concentrated anatoxin at an interval of 18 days. Their sera was tested 18 days after the first inoculation and again ten days after the second inoculation. In neither case was any antitoxin found in the serum. The experiment was not carried further, and no more work was done with a concentrated type of anatoxin.

These experiments demonstrated the great variations in the response of sheep to the inoculation of both anaculture and anatoxin, some animals developing more antitoxin than others, and again the methods used failed to detect any antitoxin in the sera of yet other sheep. The higher the antitoxin content of the serum the more resistant were the animals to the drastic methods employed to test immunity, namely, administering large quantities— 3 to 3½ litres of broth culture by the mouth and the intramuscular inoculation of culture. In animals showing 25 units of antitoxin or more per c.c. of serum the resistance to infection was complete, and sheep with at least 5 units of antitoxin in their serum per cubic centimetre, withstood attempts to infect them by feeding large quantities of culture.

The degree of resistance to infection runs parallel with the concentration of antitoxin in the serum, and resistance or

immunity appears to be obtained just as effectively by vaccination with anatoxin as with anaculture. Although immunity was produced by the single inoculation of a dose of 5 c.c. of anatoxin, larger doses—50 c.c. may be more effective. Further, a second inoculation generally increases the antitoxin content of the serum. As these experiments were designed with the purpose of gaining

TABLE III.

No. of Experiment.	*No. of Sheep.*	*Units of Antitoxin in Serum before Inoculation.*	*Inoculation.*	*Units of Antitoxin in Serum after Inoculation.*	*Method of Treating Immunity.*	*Result.*
(1)	1	None	Anatoxin, 2 inoculations each 1 c.c.	None		
	2	„	Do. do.	„		
	3	„	Do. do. 10 c.c.	„	Fed 3½ litres broth culture.	Infected and chloroformed.
	4	„	Do. do. 10 c.c.	25	Do.	Remained well.
	5	„	None	None	Do.	Died in 20 hours.
(2)	6	„	Anatoxin, 2 inoculations each 50 c.c.	25	Do.	Remained well.
	8	„	None	None	Do.	Died in 24 hours.
	7	„	Anatoxin, 2 inoculations each 50 c.c.	25	Do.	Remained well.
	9	„	None	None	Do.	Died in 24 hours, but disease not typical.
(3)	10	„	Anatoxin, 2 inoculations each of 10 c.c. Inoculated a third time, but no antitoxin demonstrated and dismissed from experiment.	None	Do.	
	11	„	Do. do. 10 c.c.	25	Do.	Remained well.
	12	„	None	None	Do.	Died 24 to 36 hours later.
(4)	13	„	Anaculture, 2 inoculations each of 50 c.c.	25	Fed 3½ litres of broth culture.	Remained well.
	15	„	None	None	Fed 3½ litres of broth culture.	Dead in 24 hours.
	14	„	Anaculture, 2 inoculations each 50 c.c.	25 5 mths. later only 5.	Inoculated intramuscularly with culture	Dead 15 hours later.
	16	„	None	None	Do.	Do.
	13	„	See above	„	Do. One week after having been fed culture.	Lame but recovered.
(5)	17	„	Anatoxin, 1 inoculation of 5 c.c.	5	Fed 3 litres of broth culture.	Remained well.
	19	„	Anaculture, 1 inoculation of 5 c.c.	Less than 5	Do.	Do.
	22	„	None	None	Do.	Dead 18 hours later.
	18	„	Anatoxin, 1 inoculation of 5 c.c.	5	Inoculated intramuscularly with culture.	Lame but recovered.
	20	„	Anaculture, 1 inoculation of 5 c.c.	Less than 5.	Do.	Dead within 15 hours.
	21	„	None	None	Do.	Do.
(6)	23	„	Filtrate anaculture, 2 inoculations of 10 c.c.	5 3 mths. later none.	Fed 3½ litres of broth culture.	Dead in 24 hours.
	24	„	Do. do.	None	Inoculated intramuscularly	Dead in 24 hours later.
	26	„	None	„	Do.	Do.
	4	„	See Experiment 1	„	Do.	Lame but recovered.
(7)	27	„	Concentrated anatoxin, 2 inoculations of 1 c.c.	„		
	28	„	Do. do.	„		

information which would be of value in planning field vaccinations to protect sheep against a natural attack of the disease, little consideration was given to the effect of more than two inoculations as it would not be practicable to inoculate sheep in the field more than twice; indeed it would be preferable if immunisation could be accomplished by one inoculation. Therefore, in the preparation of anatoxin for use in the field the toxin content of the original culture should be as high as possible, a strain of bacterium capable of potent toxin production and a suitable medium being used.

The experiments indicated that immunised sheep remained protected in some cases for at least five months, but that during this time a decline in the degree of immunity probably occurred.

The experiments held out the hope that the inoculation of sheep in the field with anatoxin will afford a means of protecting the animals against " struck." Even though some experimental animals failed to respond as well as others, and were not sufficiently protected to withstand the drastic feeding or inoculation tests, they may, nevertheless, have been sufficiently protected to withstand a natural field infection.

Field Vaccination Experiments 1931 *and* 1932.

Anatoxin was prepared in the manner already described.

In January and February of 1931, sheep on different areas of the Romney Marsh were vaccinated, some receiving one inoculation, and others receiving two inoculations of 10 c.c. each of anatoxin at an interval of ten to 14 days. In each field where vaccinated sheep were, an equal number of non-vaccinated sheep were kept as controls. Altogether approximately 4,000 sheep were under experiment, 2,000 being vaccinated animals.

It was hoped that at the end of the season the owners and shepherds would be able to supply information regarding the value of the vaccinations, but very few sheep died during the season and, although accurate figures were not kept by the shepherds or owners, it was evident that no material difference in the number of deaths in the vaccinated and control groups occurred.

Field vaccinations were again made in 1932, and in January and February, 2,048 sheep were vaccinated, two inoculations, each of 10 c.c. of anatoxin being given. In each field with the vaccinated sheep an equal number of sheep were left unvaccinated to act as controls.

During the period of the year when " struck " is expected, namely, from the middle of February to the end of May, every sheep dying in these flocks was autopsied and when found necessary a bacteriological examination was made. Excluding one or two deaths directly attributable to lambing, only 20 vaccinated

and 26 control animals died. One of the vaccinated animals presented all the characteristic lesions of "struck." This animal was examined immediately after death and culture media were inoculated with tissue from the liver, the spleen, the mesenteric lymph glands, and heart blood, but these all remained sterile. *B. paludis* was isolated from five of the control sheep and it is considered that these sheep died from "struck." In no other case was there any evidence that death was due to "struck." On account of the comparative rareness of the disease during the past two seasons, 1931 and 1932, the field vaccination experiments yielded no definite information.

General Summary.

During 1930, 75 per cent. of sheep regarded by the shepherds and owners as having died from "struck" were considered to have suffered from a *B. paludis* toxæmia or infection.

During the 1931 and 1932 seasons comparatively few sheep died on the Romney Marsh, and the examination of 63 cases of alleged "struck" showed that only a relatively small percentage were due to a *B. paludis* toxæmia or infection. It is considered likely that in seasons when mortality is high and the owners and shepherds complain of many losses from "struck," the excessive mortality is due to a *B. paludis* toxæmia or infection, but in other seasons the mortality on the Romney Marsh is little, if at all, higher than in other parts of the country where specific contagious disease is not suspected and that in such seasons deaths from *B. paludis* toxæmia or infection are very rare occurrences and of negligible economic importance.

The disease may be produced in sheep by the administering of comparatively small numbers of *B. paludis* when these are enclosed in a small, 1 or 2 c.c. gelatin capsule.

The disease was not produced in sucking lambs by feeding these with quantities of *B. paludis* broth culture along with their food. Wide variations in the composition of the diet did not affect the apparent insusceptibility of lambs to the ingestion of cultures of *B. paludis*.

Rabbits and guinea-pigs fed with relatively large quantities of *B. paludis* remained well.

Guinea-pigs have not been found suitable animals for immunisation experiments when their immunity was tested by the inoculation of cultures of *B. paludis*.

Rabbits proved suitable animals for preliminary experiments, their immunity response being gauged by the estimation of the antitoxin content of their sera and their resistance to the intravenous inoculation of toxin.

Sheep inoculated with anatoxin or anaculture showed considerable variation in their resulting immunity. Anaculture,

sterilised bacteria plus toxoid, was not found to be a superior antigen to anatoxin.

Sheep inoculated with anatoxin or anaculture and showing appreciable quantities of antitoxin in their sera withstood the oral administration of quantities of broth culture of *B. paludis* which were lethal for control sheep, and some withstood the intramuscular inoculation of quantities of *B. paludis* culture, lethal for less highly immunised sheep and for control animals.

Sheep inoculated with anatoxin or anaculture and showing little or no antitoxin in their sera did not withstand the above very severe tests.

The majority of sheep respond to the inoculation of anatoxin or anaculture by producing antitoxin. Therefore, when animals in the field are vaccinated with anatoxin or anaculture the majority may be expected to respond by producing antitoxin and to be immune to natural infection. Furthermore, it is possible that those whose serum contains insufficient antitoxin to be detected by the methods employed in these investigations, may, nevertheless, be sufficiently immune to resist a natural infection.

Field vaccination experiments during 1931 and 1932 have yielded no information because of the comparative absence of disease during these years.

Reference.

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THE TRANSMISSION OF LOUPING ILL TO FIELD VOLES.

By G. M. Findlay and C. Elton.

(*From the Wellcome Bureau of Scientific Research, London, and the Bureau of Animal Populations, Department of Zoology and Comparative Anatomy, Oxford.*)

In association with the fluctuations which occur in the numbers of certain species of wild animals such as the field vole, *Microtus agrestis*, and the Norwegian lemming, *Lemmus lemmus*, epidemics of disease are by no means rare (Elton, 1931), such epidemics, in association with lack of food, being two of the principal factors which end the phase of periodic numerical increase. Up to the present, however, such recurrent epidemics among wild animals have received but little attention.

As part of an investigation into these epidemics a preliminary study has been made of the susceptibility of the field vole to infections to which it might be exposed under natural conditions. Among such infections is louping ill, a disease caused by a filterable virus and prevalent among sheep kept on certain hill pastures, more especially in the border counties of Scotland and England. The infection, it has been shown by Macleod and Gordon (1932), is transmitted by the tick *Ixodes ricinus* L. either in its nymphal or adult form. Since ticks do not feed exclusively on sheep it is possible that voles may occasionally be bitten by ticks infected with louping ill, or when suffering from louping ill may infect clean ticks, thus serving to maintain the infection on certain pastures even in the absence of sheep. Macleod (1932) however fails to mention the vole in relation to *I. ricinus*, though in the absence of sheep it has been found on such rodents as the hare, rabbit and rat.

The Transmission of Louping Ill to Voles.

Experiments were, therefore, undertaken in order to determine whether voles are susceptible to louping ill. The voles were taken from a stock bred for several generations in the laboratory at Oxford by Mr. R. M. Ransom, under the direction of Dr. John R. Baker. They were originally derived from hill voles caught in Wales and Roxburghshire and possibly Aberdeenshire. They belong in all probability to the species *M. agrestis*, but the exact delimination of the northern vole, *M. agrestis*, and the southern form, *M. hirtus*, is not yet certain. Under laboratory conditions voles have a high viability in the absence of special epidemics.

Six voles were injected intracerebrally with a 10 per cent. suspension in broth of mouse brain infected with louping ill, each

vole receiving 0·03 c.c. Four mice were similarly injected. Death in the case of the voles occurred in four, seven, seven, eight, nine and eleven days respectively, with symptoms of tremor and hyperexcitability followed by coma. The control mice died in four, five, five and six days after inoculation. The brain of one of the voles dying on the seventh day was inoculated intracerebrally into four other voles, which died in five, six, eight and ten days after injection. Brain from the same vole was also inoculated intracerebrally into four mice, which died five, six, six and nine days after inoculation with symptoms typical of louping ill. The histological changes in the brains of these mice were also characteristic of louping ill. In a further experiment four voles received intraperitoneal injections of 0·2 c.c. of a 20 per cent. suspension of infected mouse brain in broth and at the same time intracerebral inoculations of 0·03 c.c. of a 2 per cent. suspension of starch in physiological saline. The voles died in seven, nine, eleven and thirteen days with nervous symptoms. These experiments, therefore, show that field voles may be infected with the virus of louping ill either by direct intracerebral injection or by subcutaneous inoculation, provided the brain is at the same time damaged.

Histology.

The histological changes found in the central nervous system of voles dying of louping ill are very similar to those reported by Hurst (1931) as occurring in mice. Meningeal infiltration is as a rule absent over the brain-stem and cord, though it is occasionally met with

Microphotograph of cerebrum of voles. × 150.

in the main fissures of the cerebrum and around the large vessels. The infiltrating cells consist chiefly of lymphocytes with a few large mononuclear and polymorphonuclear cells, mostly in a state of karyorrhexis. In the nervous tissue occasional vessels are surrounded by perivascular cuffs of similar cells, while evidence of microglial proliferation is found, particularly in the cerebrum. The nerve cells are also affected, all degrees of chromatolysis, vacuolation and hyperchromasia of the cytoplasm being encountered, while the nuclei are shrunken, hyperchromatic or eccentric. Neuronophagia is not infrequent. The lesions are most marked in the cerebrum, the cerebellum being as a rule almost normal. Since the changes in the ganglion cells are largely independent of the perivascular infiltration and microglial proliferation it would seem that as in the sheep, monkey and mouse the ganglion cells are directly attacked by the virus.

Discussion.

Unlike rabbits, guinea-pigs and rats, which are only very slightly, if at all, susceptible to the virus of louping ill, voles appear to resemble mice in their greater susceptibility to this virus. This susceptibility is of some interest when it is remembered that field voles are of common occurrence in hill pastures such as are known to be infected with louping ill. The importance of voles in maintaining a supply of infected ticks, as well as the role of louping ill in the genesis of periodic epidemics in voles can, however, only be determined by further investigation.

Conclusion.

The field vole, *Microtus agrestis*, is susceptible to infection with the virus of louping ill, the clinical symptoms and histological appearances resembling those found in the mouse.

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MacLeod, J., and Gordon, W. S. 1932. *J. Comp. Path. & Therap.*, **45**, 240.

TUMOUR OF THE GALL-BLADDER IN A BULLOCK.

By THOMAS JOHNSTON, M.R.C.V.S., D.V.S.M.

THE specimen described was taken from an Irish bullock slaughtered in the Glasgow abattoir. The carcase was sound and in good condition, although the liver was in an advanced state of distomatosis. This is a common enough occurrence in Irish cattle, and the case would have received no further notice had it not been observed that the gall-bladder was abnormal.

Macroscopical Appearance.—Externally, the gall-bladder was pale, firm and nodular and appeared to contain little bile. The nodules, which were confined mostly to the fundal region of the gall-bladder, were of a cystic nature with calcareous walls and cavities which communicated freely with the lumen of the bladder and contained a bile-stained mucus secretion and calcareous deposit. *Internally*, a large papillomatous growth was observed. This almost completely occluded the lumen of the gall-bladder and involved that part of the wall affected by the nodulation.

The papillæ of the growth (see fig.) were slightly flattened and varied much in shape ; some were triangular, wide at the attached end

and narrow at the free extremity ; others consisted of a narrow stalk or pedicel and an enlarged free extremity, which in many cases divided into two or more irregular, tapering or club shaped processes.

Many of the papillæ showed crenated edges. The free growing extremity of each papilla was vascular and reddish in colour. The connecting stalk was less vascular and of the same colour as the normal mucous membrane of the gall-bladder.

In the wall of the gall-bladder, there was considerable calcareous deposit, which extended into the attached end of some of the papillæ.

Microscopical Appearance of Tumour.—Each papilla showed a central core of fibrous tissue with blood vessels and a few glandular acini. Toward the surface, the glandular tissue became more abundant at the expense of the connective tissue, the latter becoming more cellular and infiltrated with lymphocytes. The lymphocytic infiltration was most marked at the growing extremity of the papilla and immediately around the acini. Many of the acini were cystic and distended with inspissated mucus secretion and cell debris; the largest of these cysts were observed macroscopically as small yellow easily enucleated areas already described. The epithelium of the acini was of the columnar type. In many of the acini there was complete obliteration of the lumen by degeneration and consequent enlargement of the lining mucous cells. Ulceration was observed at the free extremities of the papillæ.

Remarks.—The tumour described was apparently a papillary form of adenoma. No neoplasms were observed in other organs.

On the neck of the gall-bladder were observed a few nodules, which were found to be simple diverticula communicating freely with the cavity of the bladder; microscopically, the only alteration was an increase of fibrous tissue.

Note by the Editor.—It seems possible that the tumour was not an adenoma, but a papilloma or polypus.

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